Organic Chemistry

FOR

ADVANCED STUDENTS

VOLUME I

Analytical & Synthetical

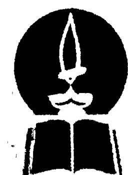
PART II

by

V. V. NADKARNY

A. N. KOTHARE

St. Xavier's College, Bombay



Lamington Road, BOMBAY 7

FIRST
PUBLISHED
1940

Printed by
K. L. N. RAO,
BOMBAY VAIBHAV PRESS
417/421, Sardar Vallabhbhai Patel Road,
Bombay 4

Published by
G. R. BHATKAL
POPULAR BOOK DEPOT
Lamington Road, Bombay 7

CONTENTS

PART ONE

PART TWO

CHAPTER VI

PLANT PIGMENTS

Introduction. In addition to carbohydrates, fats, proteins, and enzymes, there are a few other important classes of compounds that have been isolated from plants. These include the highly coloured substances called *plant pigments* and a series of complex alicyclic alcohols known as *sterols*. A brief summary of the extensive investigations carried out on the nature and structure of the plant pigments will be given below. The chemistry of these pigments has been successfully unravelled through the joint efforts of pioneer workers like Kostanecki, Willstatter, Perkin (A. G.), Robinson, Karrer.

Classification. A classification of the plant pigments is based on their location in the different parts of the plants. Thus we have :

(a) The *plastic pigments associated with the protoplasmic structure of the plants* : these comprise *chlorophyll*—the green colouring matter of all leaves, and the *carotenoids*—the yellow or brown pigments widely distributed in all kinds of plants.

(b) *The pigments from flowers and berries* which exist in solution in the cell sap, as natural glycosides. They include the bright red and blue colouring matters called *anthocyanins* and the *anthoxanthins* or flavones, which represent the pale yellow pigments. The anthocyanins and the anthoxanthins are closely associated together in nature and are also closely related to each other in structure.

Another classification, based on structural considerations has been proposed and is very useful. According to this, the pigments have been divided into :—

(a) Polyene pigments : carotenoids.
(b) Pyrrole pigments : chlorophyll.

(c) Pyrone pigments : flavones and flavonols.
(d) Pyrylium pigments : anthocyanins.
(e) Quinone pigments : rapanone, alizarine, lawsone, etc
(f) Indole pigments : indigo.

In each case, the name is derived from the basic structure present in the pigment molecule. The carotenoids, because of their solubility in fats are also known as lipochrome pigments.

Carotenoids

Introduction. Carotenoids comprise the class of natural pigments which are yellow, orange or brown and widely distributed in both the vegetable and the animal kingdom. The first member of this class was isolated by Wackenroder in 1831, as ruby red crystals from carrots and named *carotin*. Hence, Tswett proposed the class name carotenoids. The carotenoids are found in all kinds of plants and are closely associated with chlorophyll, the green colouring matter. They also bear close associations with the terpenes, sterols, lipoids etc. and are classed as 'tetraterpenes'. Several of them are recognised to be precursors of the growth-promoting vitamin A.

General Composition and Properties. The carotenoids are mostly hydrocarbons or hydroxy-carboxylic acids which contain 40 carbon atoms. The most important are the hydrocarbons; of these *carotene* and *lycopene* are the most common and plentiful. Carotenes containing less than 40 carbon atoms are known, but are less common. The pioneer researches of P. Karrer and his collaborators have established that all carotenoids are unsaturated and possess in their molecules as many as *seven to eleven conjugated double* bonds. It is this conjugated system that is the chromophore to which they owe their colour. They vary in colour and shade depending on the number of conjugated systems present in the molecules. They are insoluble in water, acids or alkalies, but soluble in chloroform, ether and fats. Because of their high unsaturation, they are easily oxidised by atmospheric oxygen; all of them may be catalytically hydrogenated to saturated compounds which are colourless.

Nomenclature and Classification. Carotenoids have been subdivided on the basis of their natural source, into (a) phyto-

carotenoids which occur in plants are *(b)* zoo-carotenoids which are found in animals. So far, about sixty carotenoids have been isolated. Structurally, they belong to four different types :

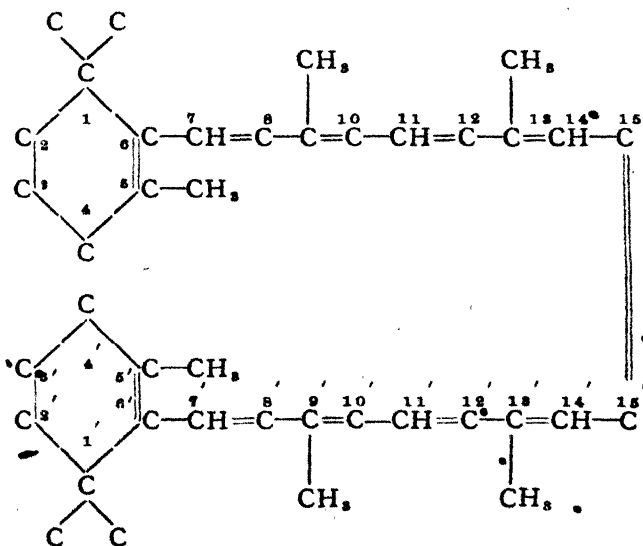
(*i*) hydrocarbons : carotenes,

(*ii*) ketonic or hydroxylic derivatives : xanthophylls, (or carotenols),

(*iii*) acidic derivatives : carotenoid acids,

(*iv*) ester derivatives : xanthophyll esters.

The carotenoids contain one or two ionone units linked together by a chain of carbon atoms united in a sequence of conjugated double bonds. The numbering of these carbon atoms has been done according to different systems. The most widely accepted system is one proposed by Karrer. It is given below :



(Kuhn and Brockmann have adopted an entirely different system of numbering the carbon atoms).

Isolation of Carotenoids. Usually the carotenoids occur as complex mixtures which cannot be separated from one another by the ordinary methods. Special methods have been developed which effect a more or less complete separation of a particular carotenoid. Some of the important methods are :—

1. PARTITION OF THE PIGMENT BETWEEN TWO IMMISCIBLE SOLVENTS:—A number of suitable mixtures of immiscible solvents, have been prepared. Usually the upper layer (epi-phase) consists of benzene, benzine, petroleum ether or a mixture of petroleum ether and ether, and the lower layer (hypo-phase) aqueous methanol, ethanol or acetone. The carotenoids distribute themselves between the two solvents in definite proportions, and by a repetition of the process, a quantitative separation of the pigments is effected. It has been found that the carotenes containing forty carbon atoms are concentrated in the upper layer while the carotenols *e. g.* xanthophyll pass into the lower layer. Usually, petroleum ether and 90% methanol are used as the immiscible solvents. The carotenes and xanthophyll esters are concentrated in the epi-phase, while the xanthophylls and the carotenoid acids are concentrated in the hypo-phase. The epi-phase pigments are then saponified and subsequently separated by partition. The separation of the hypo-phasic pigments is effected by extraction of the carotenoid acids with dilute alkali.

2. THE CAPILLARY METHOD:—This method is based on the capillarity of the solution of the carotenoids. A strip of filterpaper is dipped into a solution of the carotenoids when different zones of colour and breadth corresponding to different carotenoids present, appear on the paper. They can be subsequently tested by chemical and spectroscopic methods.

3. TSWETT'S CHROMATOGRAPHIC METHOD:—In this method, the carotenoid is selectively adsorbed by a solid like calcium carbonate, calcium hydroxide, magnesium oxide, aluminium oxide or basic lead acetate. A glass tube drawn to a fine bore at the lower end and connected to a suction flask is filled with one of the above solid adsorbents. A solution of the carotenoid in benzene or carbon disulphide is then allowed to percolate slowly through the tube. A fixation of the carotenoids in definite zones, in the order of their relative adsorbability takes place in the adsorbent. A

further separation of the zones may be effected by refilling the tube with pure solvent. This is called the development of the chromatogram. The different zones thus formed are then eluted by using solvents which possess greater solvent action, or they can be mechanically cut out. The pigment corresponding to each zone is further examined chemically, colorimetrically and spectroscopically.

The adsorbability of the carotenoids depends on the structure of the molecule. The hydrocarbons are the least adsorbed while adsorption increases with the number of hydroxyl groups in the molecule. The number of double bonds present in the molecule also determines the adsorbability, which falls with the decrease in the number of the double bounds.

The effectiveness of the separation depends to a great extent on the choice of a particular adsorbent and solvent. Calcium hydroxide or aluminium oxide are found to be the most suitable for hydrocarbons, while the alcohols are most effectively separated by the use of calcium carbonate. Recently, the use of magnesium oxide and petroleum ether as adsorbent and solvent respectively has been recommended by Strain.

The above method first initiated and developed by Tswett can be regarded as the most satisfactory method and constitutes the most valuable contribution to the study of the chemistry of carotenoids next to spectroscopy.

Detection of the Carotenoids. A number of methods based on colour reactions and spectroscopic data have been developed for the detection of carotenoids. The following are some of the most important tests applied for this purpose.

1. CARR-PRICE COLOUR REACTION :—When a carotenoid is brought into contact with antimony chloride in chloroform solution a deep blue colouration is developed. The reaction is also used for the quantitative estimation of the carotenoids. For this purpose, the Lovibond tintometer has been employed and the results are expressed in B. V. S. (Blue values) or in terms of the C. L. O. units. A compound possesses one C. L. O. unit when 20 milligrams of it in one of Carr-Price antimony chloride solution gives the same colour as 10 Lovibond B. V. S.

2. SULPHURIC ACID :—The solid carotenoid is dissolved in ether or chloroform and concentrated sulphuric acid (about 85 per cent) added slowly so as to form two distinct layers. An intense blue colour is obtained; the shade of the colour developed depends on the number of conjugations in sequence. Thus, Kuhn and Winterstein have given the following interesting results in the case of diphenyl-polyenes. $C_6H_5—(CH=CH)n—C_6H_5$.

Value of n	*Colour produced*
n=1 or 2	no colour
n=3	yellow–orange
n=4	red
n=5	violet–red
n=6	blue
n=7 or 8	blue–green

These results indicate that the presence of six conjugations in sequence is necessary for the production of blue colouration.

In addition to these tests, a number of colorimetric methods have been evolved. They involve the comparison of the colour produced by the carotenoid in a suitable solvent with that of a standard solution.

3. SPECTROSCOPIC METHODS :—The absorption spectrum of a carotenoid is quite characteristic and can, therefore, be used to distinguish and identify a carotene. The absorption spectra of carotene, lycopene, and xanthophyll have the same general shape which indicates a close resemblance in their chemical structures. The absorption curves for a large number of carotenes have been obtained by numerous investigators, and have been of immense value in the study of the chemistry of carotenes.

General Methods Employed in the Determination of the Structure of Carotenoids. A number of physical properties and chemical reactions have been utilised for the structural elucidation of the carotenoids. The physical properties that have been of great help are :—

COLOUR :—Carotenoids very in their colour from bright yellow to red or deep blue. The particular shade is determined by the number and distribution of the system of the conjugated

double bonds in the molecule. Kuhn and Winterstein who have carried out extensive investigations on the simple diphenyl polyenes C_6H_5—$(CH=CH)$n—C_6H_5 have reached important conclusions, regarding the influence of the number of conjugated systems on the appearance of colour. The conclusions are: (1) The depth of colour increases with the number of conjugation in immediate and uninterrupted sequence, wherein the conjugations of the benzene ring also figure. (2) The carbonyl group, whether present as a ketonic or carboxylic group contributes a double bond. (3) An uninterrupted sequence of 5 or 6 double bonds is required to produce a visible colour. These data have been used to determine the approximate number and distribution of the unsaturation in the molecule.

POLARIMETRIC DATA :—These have been very useful in locating the centres of asymmetry in the molecules of the optically inactive and active carotenoids.

Spectroscopic evidence :—The study of the different spectra, *e. g.* absorption spectra, Raman spectra, Xray spectra fluorescence and emission spectra, has proved of immense help in the elucidation of the constitution of many carotenoids. The absorption bands of two carotenoids with the same number and distribution of the conjugations are found to coincide. Thus, carotene, kryptoxanthin and zea-xanthin can hardly be distinguished from one another spectroscopically. It is found that slight alteration in the chromophoric part of the carotenoid molecule, causes a corresponding change in its absorption spectrum. The results of the application of spectroscopic methods have enabled to deduce probable constitutions of the carotenoids.

OTHER EVIDENCE :—In addition to these, a large number of reactions have been employed to throw light on the structural chemistry of the carotenoids. Thus, we have :—

(a) Action of heat :—Application of heat causes deep-seated changes in the carotenoid molecules. The relatively simple products of decomposition, so formed, help to elucidate the structure of the complex carotenoids. As a rule the methyl groups survive this treatment and thus, their relative positions in the decomposition products enable one to fix their location in the original molecule. Many a carotene, on such heat-treatment, yields the following :— toluene, *m*-xylene, *m*-toluic acid and 2-6 dimethyl naphthalene.

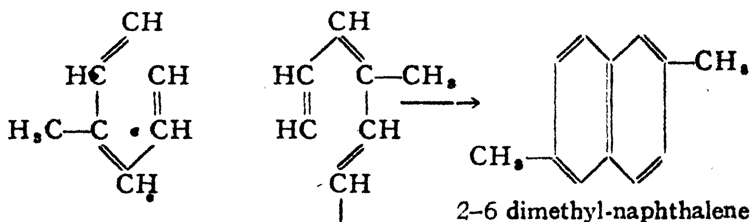
The formation of such decomposition products has been satisfactorily accounted for by Kuhn. The different products arise from different parts of the carotenoid molecule.

The $-CH-CH=CH-\underset{\displaystyle CH_3}{\underset{|}{C}}=CH-CH-$ chain gives rise

to toluene by cyclisation, while a similar cyclisation of the portion $-CH-\underset{\displaystyle CH_3}{\underset{|}{C}}=CH-CH=CH-\underset{\displaystyle CH_3}{\underset{|}{C}}-$ would produce *m*-xylene. The

carotenoid acids, on the other hand, with the grouping $CH=CH-\underset{\displaystyle CH_3}{\underset{|}{C}}=CH-CH=CH-COOH$, would yield *m*-toluic acid. A

double cyclisation of the middle portion of the carotenoid molecule would result in the formation of the naphthalene system :—



2–6 dimethyl-naphthalene

(*b*) *Action of potassium permanganate* :—By this reagent, carotenoids are decomposed into various oxidation products which can be readily identified. β-Carotene, thus, gives on oxidation, geronic acid, α-α-dimethyl glutaric acid and α-α-dimethyl succinic acid; these results indicate the presence of β-ionone nucleus in β-carotene.

Oxidation of the carotenoid by alkaline permanganate proceeds in such a way that the $=CH-\underset{\displaystyle CH_3}{\underset{|}{C}}=$ group is converted into acetic acid. The reaction can therefore, be employed to determine the number of these groups and hence, the number of the isoprene units in the carotene molecule. (Each molecule of acetic acid

formed indicates the presence of one isoprene unit.) However, it attacks incompletely the grouping :—$CH_2—\underset{\displaystyle CH_3}{\overset{|}{C}} =$.

(c) *Chromic acid* :—Karrer, Kuhn and their collaborators have shown that the results of oxidation of a carotenoid by chromic acid are more reliable, especially for the determination of the lateral methyl groups. Usually, the first step in the oxidation of a carotenoid by chromic acid is the formation of a glycol, which takes place on the terminal cycles. The glycol is further oxidised to a diketone with the splitting of the chain at this point. The diketones so obtained are often the 1–6 diketones that readily suffer cyclo–dehydration under the influence of alkali to cyclo–pentene derivatives. Chromic anhydride is also very useful; it is used for the determination of C-methyl groups in organic compounds (Kuhn-Roth method). Unlike alkaline $KMnO_4$ this reagent attacks completely both the groups :

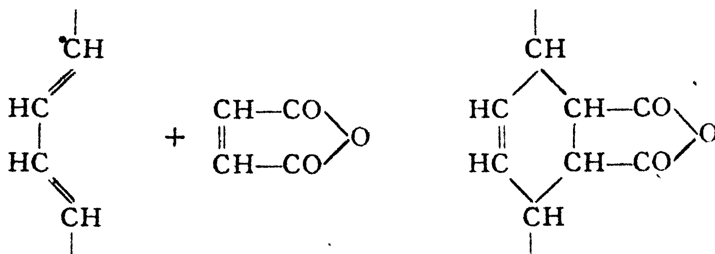


present in β-carotene.

(d) *Ozone* :—This reagent has proved very fruitful in the investigation of the carotenoids. The ozonides are readily formed and on hydrolysis, give products which enable the determinution of the terminal groups to be effected with ease and readiness. Groups : $(H_3C)_2C=$, in the carotenoid molecule appears as acetone which can be estimated iodometrically. The constitution of β-carotene is based chiefly on the results of ozonolysis of the molecule. It is by such results that the presence of two β-ionone units has been indicated in β-carotene.

(e) *Addition reactions* :—The carotenoids are highly un-saturated compounds and the foregoing reactions have been employed to determine the location of the double bonds. The exact degree of unsaturation in the molecule is established by addition reactions. The common addenda used are hydrogen (catalytic), halogens, chiefly bromine in chloroform solution, and maleic anhydride. The adducts formed with maleic anhydride in benzene solution are often crystalline compounds with sharp melting-points and hence, are of great significance and importance.. The

number of conjugations in the molecules as indicated by other methods, is confirmed by the nature of the maleic anhydride adduct formed. One molecule of the anhydride is added per one conjugation present in the molecule :—



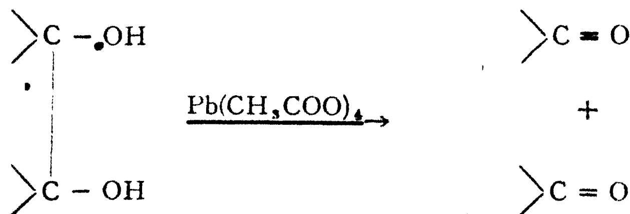
(*f*) *Methyl-magnesium-iodide* :—Zerewitinoff has developed a method for the estimation of hydroxyl groups. It consists in the action of methyl-magnesium-iodide on the compound containing the hydroxyl, in anhydrous solvents :—



$$ROH + CH_3MgI \longrightarrow R-OMgI + CH_4$$

The methane formed is estimated and gives a measure of the hydroxyl groups in the molecule. This method has been successfully applied to carotenoids.

(*g*) *Lead tetra-acetate* :—This reagent finds use as a mild oxidising agent and is of special value for the smooth oxidation of 2-glycols.



Criegee has evolved a method which uses the above reaction for the determination of the location of hydroxyl groups in a carotenoid.

(*h*) *Hydroxylamine* :—Carotenoids containing carbonyl groups have been classified by Kuhn and Brockmann according to the

ease with which they react with hydroxylamine. Thus we have :—

(a) Carotenoids with the groupings—$(CH=CH)n—CHO$. or —$(CH_2)n—CO—CH_3$ react readily.

(b) Carotenoids with conjugated keto-group of the type

$$=\underset{}{\overset{CH_3}{\overset{|}{C}}}—CO—CH_3$$

as in β-carotenone react with great difficulty.

(c) Carotenoids with the keto-grouping such as $>CH—CO—CH=CH—$ do not react at all.

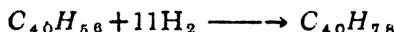
Carotenes

The first carotene was isolated by Wackenroder from carrots. Carotenes occur in green leaves closely associated with chlorophyll and in carrots. The yellow colour of many roots *e.g.* carrots and of fats like butter is due to the presence of carotenes. The carrots still continue to be the most convenient source of the carotenes, because they contain them in the highest percentage and as the sole colouring matter, which makes their isolation simpler and cheaper.

The dried carrot powder is extracted with petroleum ether, and the concentrated extract treated with CS_2. Alcohol is then added carefully to remove the colourless impurities first and the carotenes are finally precipitated. The crude carotenes thus obtained are dissolved in CS_2 and precipitated with alcohol and finally crystallised from petroleum ether. They have been further separated by chromatography into three isometric carotenes which are designated by the Greek letters α, β and γ. The α-isomer is soluble in petroleum ether. It is optically active and dextro-rotatory. Its melting-point is 183°. β-Carotene, on the other hand, is optically inactive and melts at 187°. The proportion of the α- and β-isomer varies in different plants. The β-isomer is the most common and most plentiful. It is the one that has been investigated very thoroughly.

Molecular Composition and structure of β-Carotene. The molecular composition is given by the formula $C_{40}H_{56}$ (Willstatter). The structural formula is based on the following analytical evidence collected by P. Karrer and co-workers.

THE NATURE, AMOUNT AND LOCATION OF UNSATURATION IN THE MOLECULE :—(a) On catalytic reduction, with hydrogen in presence of platinum, each molecule of carotene takes up 11 molecules of hydrogen giving a saturated hydrocarbon, $C_{40}H_{78}$ per hydro-vitamin A which is colourless.



$$C_{40}H_{56} + 11H_2 \longrightarrow C_{40}H_{78}$$

β-Carotene, therefore, must contain eleven double bonds and two nuclei.

(b) β-Carotene forms with maleic anhydride in benzene solution, a crystalline adduct, $C_{40}H_{56}(C_4H_2O_3)_5$ m.p. 285°-86°; this indicates that there are five conjugations in the molecule.

NATURE OF CARBON FRAMEWORK :—This is revealed by the study of the oxidation products of the molecule.

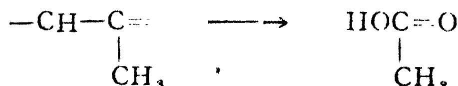
(a) When exposed to dry oxygen, β-carotene takes up 35 per cent of its weight of the oxygen gas and gives a distinct smell of violets. The development of the violet smell indicates the presence of ionone nucleus (cyclohexenyl units) in the molecule.

(b) Ozonolysis of β-carotene gives *geronic acid :* one molecule of β-carotene gives two molecules of geronic acid. Hence there must be two β-ionone nuclei.

Lastly, oxidation of β-carotene in benzene with $KMnO_4$ in the cold gives β-ionone.

Probably the two ionone nuclei are separated by a polyene system containing nine double bonds, the remaining two double bonds being present in the two ionone nuclei, one in each. Further, the ionone units together contain twenty carbon atoms; hence, the remaining twenty carbon atoms must be present as four isoprene units (C_5H_8). This suggests the presence of four lateral methyl groups.

(c) Permanganate (alkaline) oxidation of β-carotene actually gives four molecules of acetic acid :—



$$\underset{\displaystyle CH_3}{-CH-\overset{}{C}=} \longrightarrow \underset{\displaystyle CH_3}{HOC=O}$$

Hence, the presence of four lateral methyl groups is established.

Kuhn, as a result of chromic anhydride oxidation has shown that two more groups of the type :—CH_2—C = are present in

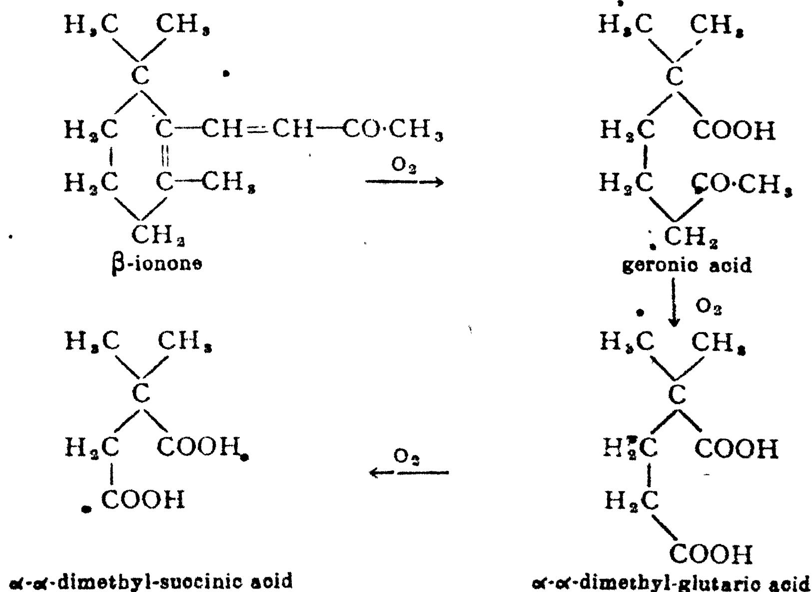


carotene.

(*d*) With potassium permanganate, under different conditions, β-carotene gives together with β-ionone, (*i*) geronic acid (ii) α-α-dimethyl-glutaric acid, (*iii*) α-α-dimethyl-succinic acid and (*iv*) dimethyl malonic acid.

These decomposition products have been satisfactorily accounted for as follows :—

β-Carotene is first converted into β.ionone which is successively oxidised to geronic acid, α-α-dimethyl-glutaric acid and α-α-dimethyl-succinic acid :—

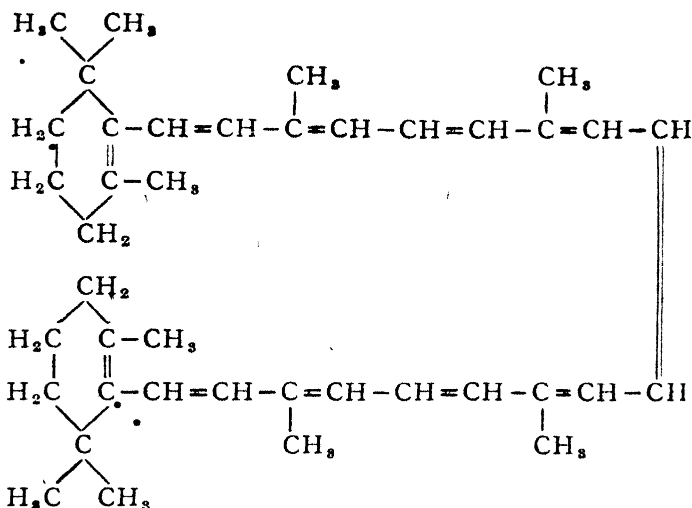


From the above evidence it follows that β–carotene is built up of :—

(*a*) two β-ionone units,

(*b*) a polyene chain separating the two ionone units ; the chain contains four lateral methyl groups, *i. e.* is made up of four isoprene units.

He β-carotene may be best represented by :—

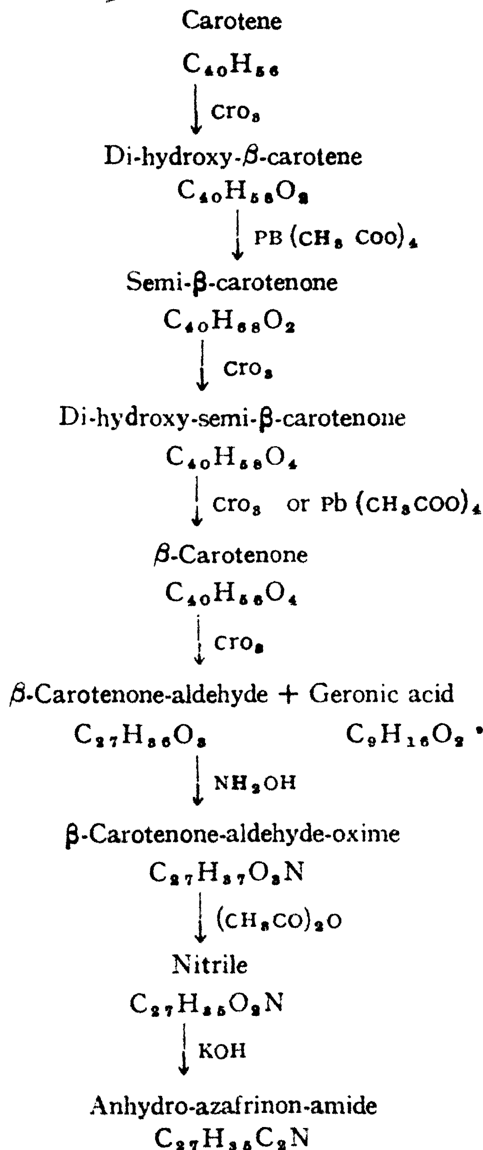


This structure is, further, in good agreement with the fact that β-carotene is a progenitor of vitamin A. In the animal body β-carotene is changed into vitamin A. Vitamin A is, therefore, a derivative of β-carotene. Vitamin A aldehyde has now been obtained by oxidation of β carotene with MnO_2, H_2O_2 etc., and this compound is as active as the vitamin A (alcohol). It is believed that in the body, a similar oxidation takes place. Lastly, the oxidation of carotene with OsO_4 gives vitamin A.

The four isoprene units in the molecule are present in such a way that the first two (from either ionone unit) are joined head to tail, then there is inversion and the remaining two are joined head to tail again; if all the four isoprene units were joined head to tail without inversion, two molecules of β-ionone from one of carotene, would not have been formed.

Lastly Kuhn and Brockmann have carried out extensive investigations involving degradation and synthetic reactions, and thus, revealed the structural complexities of the β-carotene molecule. β-Carotene is, systematically converted into anhydro-azafrinonamide, which is also obtained from azafrin. Hence, the aldehydes and acids obtained by oxidation of azafrin definitely establish the structure of the, polyene chain, separating the two

ionone units in the carotenoid. These relations have been represented below :—



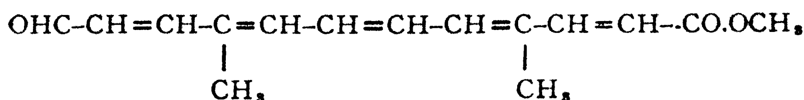
Now, azafrin can be readily converted into the identical anhydro–azafrinon–amide. β–Carotene, therefore, contains the same

polyene chain as in azafrin which is revealed by the study of the oxidation products of azafrin. We have :—

Azafrin

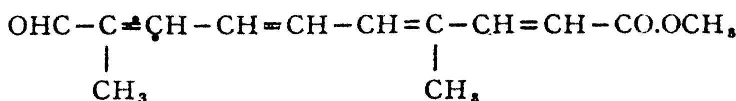






(methyl–3.8–dimethyl–decapentene–1–al–ω–carboxylate)

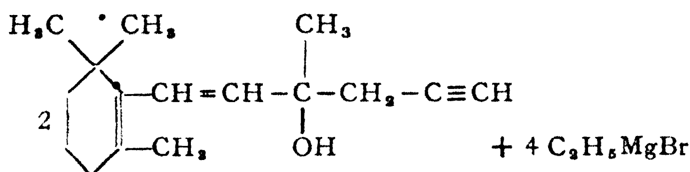


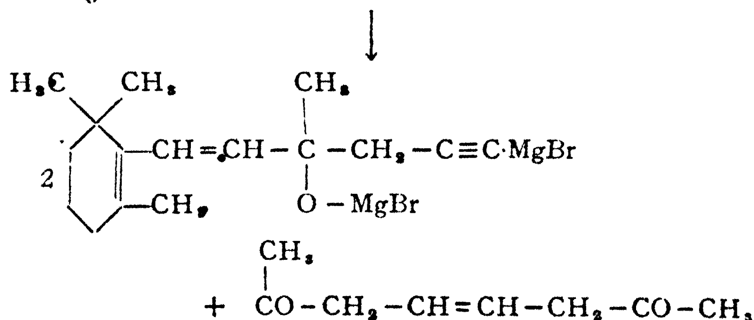


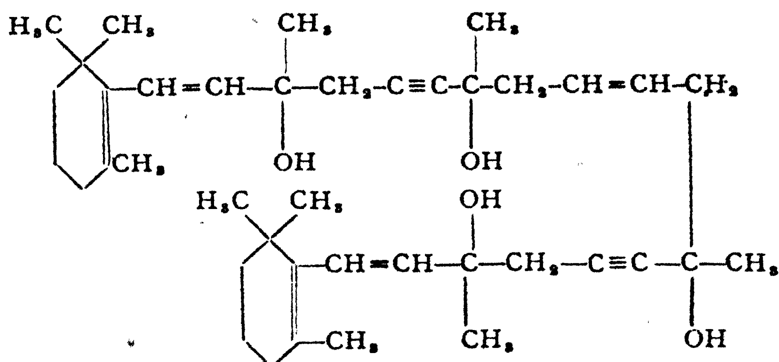
(methyl–1.6–dimethyl–octa–tetraene–1–al–8–carboxylate)



Recently a synthesis of β–carotene by P. Karrer and Eugster has been reported. (It is a $C_{16}+C_8+C_{16}$ Synthesis.)

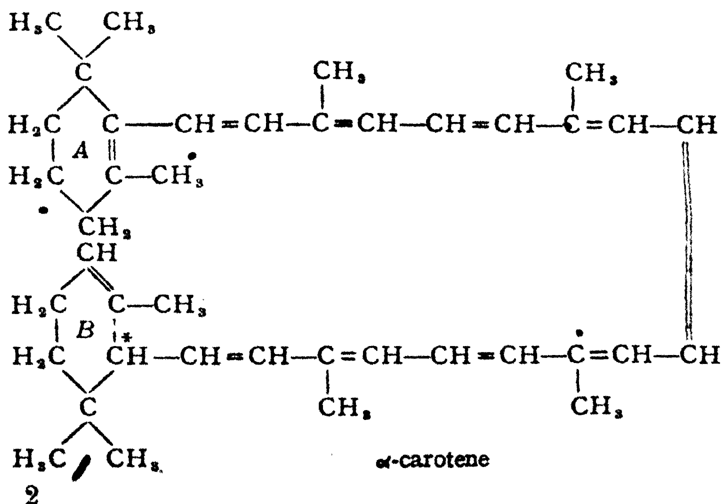






The C≡C bonds are then selectively reduced to C=C. The tetrol thus obtained is dehydrated with *p*-toluene sulphonic acid to give β-carotene.

α-Carotene. It is a structural isomer closely associated with β-carotene. Probably it differs from the latter in the position of the double bond in one of the ionone nuclei. On oxidation, α-carotene gives one molecule of geronic acid and one molecule of iso-geronic acid. (β-Carotene under the same conditions gives two molecules of geronic acid). Hence, α-carotene may be represented by :—



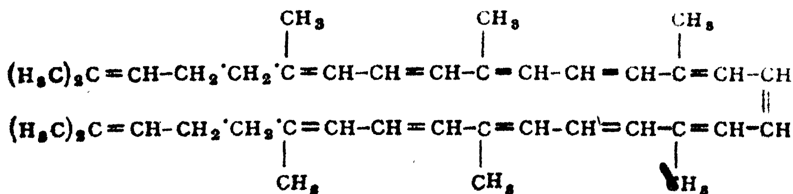
In the formula, A is the β-ionone unit present in β-carotene while B is the α-ionone unit. The β-ionone unit produces geronic acid while iso-geronic acid would result from α-ionone. The carbon atom marked with an asterisk in the α-ionone unit is asymmetric and hence, the optical activity of α-carotene.

Lycopene. It is present in tomatoes and is responsible for its red colour. Willstatter isolated it from tomatoes and determined its formula as $C_{40}H_{56}$. Thus, it is isomeric with the α-and β-carotenes. Its structural formula has been evolved from the following analytical evidence.

Nature of unsaturation :—It can be catalytically hydrogenated to a colourless saturated paraffinoid hydrocarbon $C_{40}H_{82}$. These results indicate the presence of thirteen double bonds. The carotenes with the same composition contain only eleven. It follows, therefore that lycopene does not contain cyclic units.

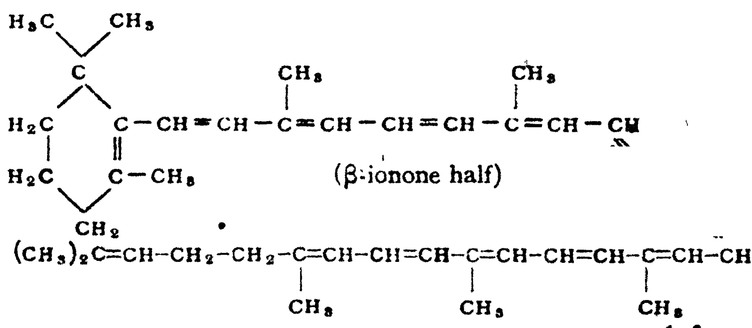
The carbon framework :—This is arrived at by the results of oxidation with *(i)* ozone. *(ii)* chromic acid and *(iii)* potassium permanganate. *(i)* On ozonolysis, lycopene gives two molecules of acetone. Thus, two terminal isopropylidene groups are indicated. *(ii)* With chromic acid, six molecules of acetic acid are formed. These results show that there are six lateral methyl groups between the two isopropylidene terminal groups. Under certain conditions methyl-heptenone is also formed. *(iii)* Oxidation with potassium permanganate gives succinic acid and acetic acid.

The above decompositions can be satisfactorily accounted for, by the following formula proposed by P. Karrer :—



Thus, lycopene is built up of eight isoprene units and can be divided into two symmetrical halves. Recently, a hydrocarbon very similar to perhydro-lycopene has been synthesised from phytol. The latter synthesis is in good agreement with P. Karrer's formula. R. Kuhn has also independently corroborated the above formula by a series of decomposition reactions.

γ–**Carotene.** It is mono-cyclic. It is built up of two halves: one half is that of β–carotene and the other is that of lycopene.



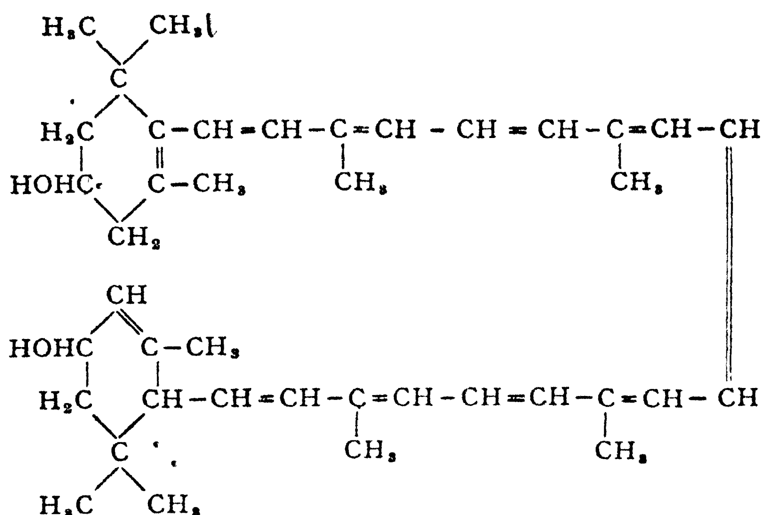
Carotenols

Plant pigments which are hydroxy derivatives of carotenes are known. They are called phyto-xanthins. The most common and typical ones are xanthophyll and zea-xanthin.

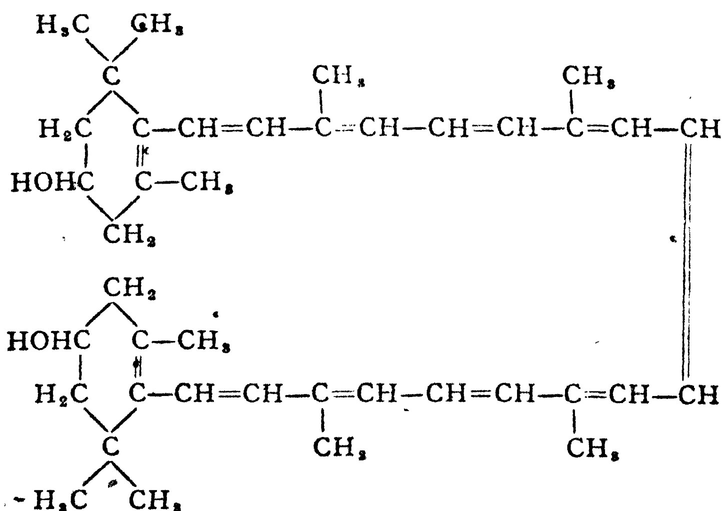
Xanthophyll (Lutein). It occurs in green leaves of all plants closely associated with chlorophyll. Its composition is $C_{40}H_{56}O_2$; it forms ruby red crystals and melts at 195°. It contains two oxygen atoms more than the carotenes. They are present as two hydroxyl groups, as ascertained by the Zerewitinoff's method.

Relation to α-carotene :—On oxidation, xanthophyll gives the same decomposition products as α-carotene. It is, thus, a dihydroxy derivative of α-carotene. The two hydroxyl groups are present on the ionone units, one on each.

Hence the following structure has been assigned to xanthophyll.



Zea-xanthin :—$C_{40}H_{56}O_2$ is isomeric with xanthophyll. It is the pigment of maize and is also found as ester in some fruits and flowers. It has been shown to be the dihydroxy derivative of β-carotene; as in the case of xanthophyll, two hydroxyl groups are present, in the two β-ionone units, one in each. The positions are indicated by the non-formation of dimethyl glutaric acid, on oxidation with potassium permanganate. Its structure has been formulated as :—



Other oxygenated carotenoid pigments, related to the carotenes are capsanthin $C_{40}H_{58}O_3$ and astacin $C_{40}H_{48}O_4$. The former is the pigment of paprika, and gives the same decomposition products as the carotenes and probably contains the same carbon framework. However, it is a ketone. Astacin has been shown by Kuhn to be a tetra-keto-derivative of β-carotene. Each β-ionone unit contains keto-groups in positions 4, 4′, 5, 5′.

Chlorophyll

Introduction. The leaves of all plants irrespective of their botanical classification contain four pigments. Two of them are the yellow pigments, carotenoids—carotene and xanthophyll which have been already dealt with. The other two are the green colouring matters now called chlorophyll *a* and *b*. The two chlorophylls are highly complex molecules containing carbon, hydrogen, oxygen, *nitrogen* and *magnesium*. The other complex organic compounds containing metallic elements are *haemoglobin*, the red colouring matter of blood, which contains *iron and haemocyanine*, the blue colouring matter of blue blood of octopus, which contains *copper*. It is quite significant that these three complex compounds possess the same fundamental structure and play an equally important rôle in the life processes of plants and animals.

Extraction of Chlorophyll from the leaves. The leaves are dried and powdered; the natural pigments are then extracted from the powdered material by the use of organic solvents like ethanol (85%), acetone (80%) or ether. Usually, a slightly moist solvent is employed. The yellow pigments are then separated from the green colouring matter by chromatographic adsorption. The pigments of the green leaves present in a petroleum ether extract are filtered through a column of adsorbent chalk. The green colouring matter is adsorbed at the top of the column and forms a distinct zone separated by the zones of yellow pigments. Further purification of the chlorophylls may be effected by distribution between two suitable immiscible solvents.

The green colouring matter obtained by the above method is an amorphous body which is a mixture of two compounds : (*i*) chlorophyll *a*. a bluish-green substance with the composition

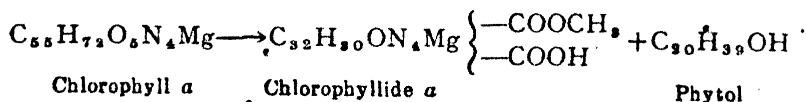
$C_{55}H_{72}O_5N_4Mg$ and *(ii)* chlorophyll *b*, a yellowish-green solid with the composition $C_{55}H_{70}O_6N_4Mg$. The former predominates in the mixture.

Willstatter has separated the chlorophylis *a* and *b* from each other by distributing them between petroleum ether and 90% methanol. The *a* isomer is concentrated in the petroleum ether while the methanolic layer contains the *b* isomer. Lately Winterstein and others have separated the two chlorophylls by chromatographic analysis, using powdered sugar as the solid adsorbent.

The nettles constitute a suitable source of chlorophyll, specially because they contain only a little chlorophyllase. The latter is an enzyme that splits the chlorophyll molecule.

Constitution of Chlorophyll. A large amount of analytical and synthetical work has been carried out by Willstatter and his school and more recently, by Hans Fischer in Germany which throws much light on the constitutional problem. The compound has been subjected to many degradative reactions, which involve the use of acids, alkalies, oxidising agents and reducing agents. An attempt will be made here, to classify the various reactions, and their decomposition products and to show their mutual relationships to one another and to the parent compound, chlorophyll.

ACTION OF ALKALI :—The hydrolysis of chlorophyll *a* and *b* with alkalies can be effected in progressive stages. In the cold, at the ordinary temperature, chlorophyll (*a* and *b*) is changed into chlorophyllide (*a* and *b*).

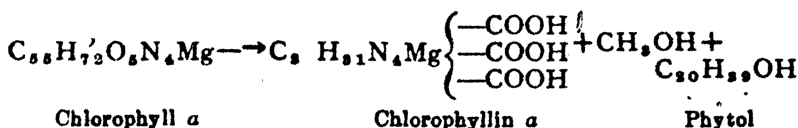


$$C_{55}H_{72}O_5N_4Mg \longrightarrow C_{32}H_{30}ON_4Mg\begin{cases}-COOCH_3\\-COOH\end{cases} + C_{20}H_{39}OH$$

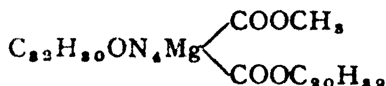
Chlorophyll *a* Chlorophyllide *a* Phytol

Similarly, chlorophyll *b* is converted into chlorophyllide *b* and phytol. Chlorophyllide *b* is further hydrolysed by alkali to give chlorophyllin *a* and CH_3OH

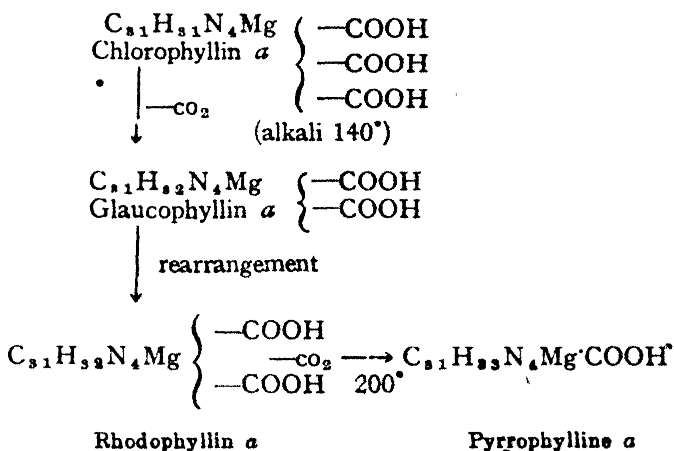
Thus chlorophyll *a* is finally converted into chlorophyllin a, methyl alcohol and phytol.



Chlorophyll *b* similarly gives the corresponding chlorophyllin *b*. Hence chlorophyll a is a diester :



The Chlorophyllin *a*, on further treatment with alkali at high temperature 140 to 200°, undergoes progressive decarboxylation accompanied by rearrangement reactions.



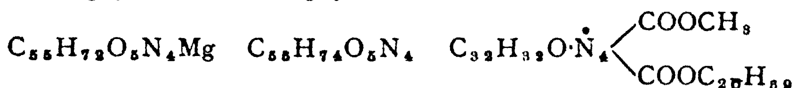
The carboxylic acids formed as above and which still contain magnesium are together called *phyllins*. They are often named after their colour, *e.g.* glaucophyllin is blue and rhodophyllin is red. The phyllins, on heating with soda-lime, are converted into *aetiophyllin* : $C_{31}H_{34}N_4Mg$, which contains no carboxyl group. Heating with alkali, also causes another change : a CH_2 group is replaced by H. Hence two different pyrrophyllins differing from each other by a–CH_2 group are known.

Chlorophyll and its derivatives are usually identified by their absorption spectra. Willstatter has developed a method of separatio

and identification of these derivatives. It consists in extracting them from ether solution with HCl of different concentrations. Chlorophyll derivatives are thus characterised by what is called "*a hydrochloric acid number.*" It represents the percentage concentration of HCl which extracts two-thirds of the compound from an equal volume of an ether solution.

ACTION OF ACIDS :—The action of weak acids *e.g.* oxalic acid on chlorophyll and the related compounds, consists in the elimination of the magnesium atom and its replacement by two hydrogen atoms. Thus, chlorophyll *a* and *b*, on treatment with acids are converted into olive green compounds. They contain no magnesium in their molecules. They are more basic than chlorophyll. The ester linking however is not affected.

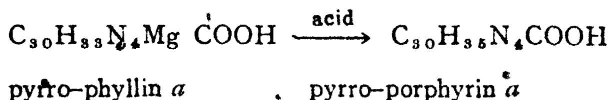
Chlorophyll *a* ⟶ Phæophytin *a*



$C_{55}H_{72}O_5N_4Mg$ $C_{55}H_{74}O_5N_4$ $C_{32}H_{32}O{\cdot}N_4\left\langle\begin{array}{l}COOCH_3\\COOC_{20}H_{39}\end{array}\right.$

Chlorophyll *b* ⟶ Phæophytin *b*

The phæophytins *a* and *b* are weakly basic compounds. The chlorophyllins (*a* and *b*) also give phytochlorins or phytorhodins. Similarly, the *phyllins* also, under the influence of acids, lose their magnesium content and are converted into the corresponding compounds, called *porphyrins*. They are amphoteric in nature.

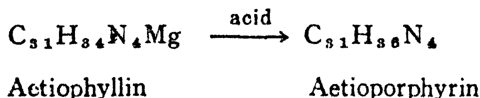


$$C_{30}H_{33}N_4Mg\ COOH \xrightarrow{acid} C_{30}H_{35}N_4COOH$$

pyrro-phyllin *a* pyrro-porphyrin *a*

Another pyrroporphyrin with C_{31} is also known.

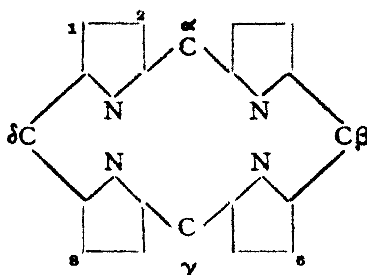
Also



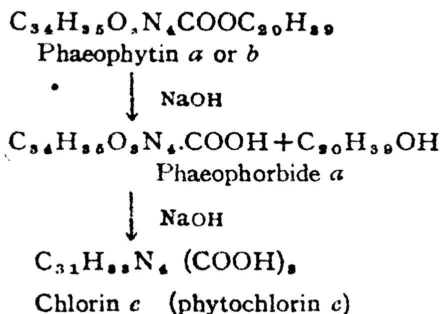
$$C_{31}H_{34}N_4Mg \xrightarrow{acid} C_{31}H_{36}N_4$$

Actiophyllin Aetioporphyrin

Porphyrins are the derivatives of the fundamental compound *porphin*, built up of four pyrrole nuclei to form a closed structure :



The phæophytins, phytochlorins phytorhodins, and porphyrins can be decomposed by alkali in progressive stages as the phyllins. Thus, we have :—



$C_{34}H_{35}O_{3}N_{4}COOC_{20}H_{39}$
Phaeophytin *a* or *b*

↓ NaOH

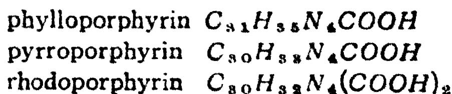
$C_{34}H_{35}O_{3}N_{4}.COOH + C_{20}H_{39}OH$
Phaeophorbide *a*

↓ NaOH

$C_{31}H_{33}N_{4}\ (COOH)_{3}$
Chlorin *e* (phytochlorin *e*)

The porphyrins are characterised by the possession of great absorption in the U. V. This has been of great use in the characterisation and identification of decomposition products of chlorophyll.

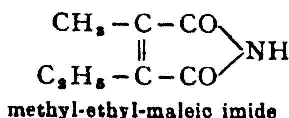
The alkaline degradation of phytochlorin *e* at high temperatures, thus gives rise to a series of 'porphyrins'. The important ones are :



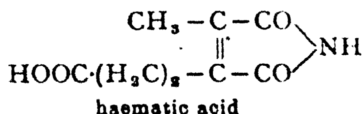
phylloporphyrin $C_{31}H_{35}N_{4}COOH$
pyrroporphyrin $C_{30}H_{33}N_{4}COOH$
rhodoporphyrin $C_{30}H_{32}N_{4}(COOH)_{2}$

On distillation with soda-lime, both the phyllo and the pyrro-porphyrins suffer decarboxylation and yield phylloaetioporphyrin ($C_{31}H_{36}N_{4}$) and pyrro-aetio-porphyrin, ($C_{30}H_{34}N_{4}$) respectively. The phyllo-compound can be converted into the pyrro-compound by heating with $NaOC_{2}H_{5}$.

The oxidation of phylloporphyrin with Caro's acid or CrO_3 or lead peroxide gives: (*a*) methyl–ethyl–maleic imide in more than one molecular proportion and (*b*) haematic acid (one molecular proportion). The structural formulas for methyl–ethyl–maleic imide and haematic acid are:



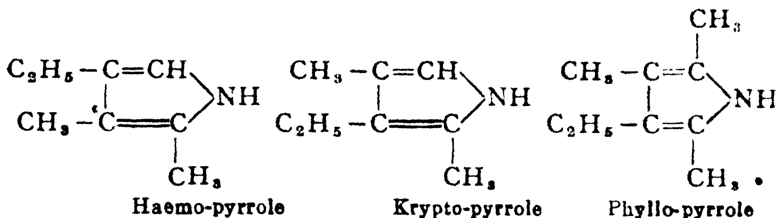
methyl-ethyl-maleic imide



haematic acid

These results, thus, clearly indicate the presence of pyrrole nuclei in porphyrins; two of them carry CH_3 and C_2H_5 group and the other CH_2-CH_2-COOH group.

The reduction of porphyrins with con HI and glacial acetic acid gives a mixture of different pyrroles. The following have been isolated:



Haemo-pyrrole Krypto-pyrrole Phyllo-pyrrole

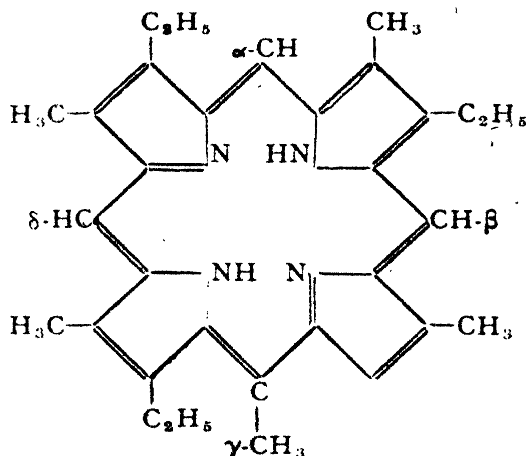
Thus, the presence of substituted pyrrole nuclei in the porphyrins is definitely indicated, in a independent way.

Fischer then proceeded to establish the constitutions of phyllo-porphyrin and pyrro-porphyrin. He synthesised the eight possible tetramethyl-triethyl porphin propionic acids, but none was found to be identical with pyrro-porphyrin; on the other hand, the latter contained C_2H_4 less than any of the synthetic products. Pyrro-porphyrin was therefore acylated and the acetyl group (—CO—CH_3) converted into the ethyl group (C_2H_5). The product obtained was found to be 1,3,5,8 tetramethyl, 2,4,6 triethyl-porphine-7 propionic acid. Hence pyrroporphyrin must be 1,3,5,8 tetramethyl-diethyl porphin-7 propionic acid. The positions of the two ethyl groups were settled as follows:

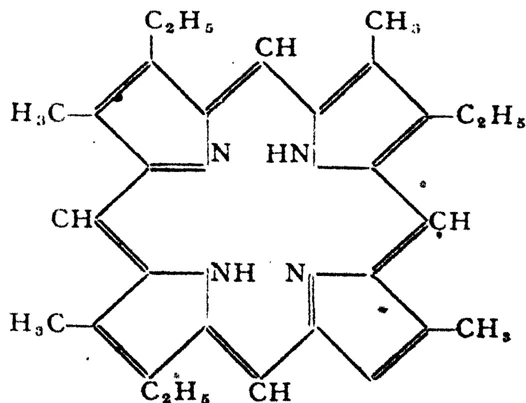
Both the rhodo-porphyrin and phyllo-porphyrin were synthesised. The former was a dicarboxylic acid and the relative

positions of the two groups —COOH and $—CH_2—CH_2—COOH$ were indicated to be in close proximity, by the absorption spectra. The extra $—CH_3$ group in phyllo-porphyrin was shown to be in the γ-position.

Pyrro-aetioporphyrin was thus shown to be 1,3,5,8 tetramethyl —2,4,7 tri-ethyl porphyrin (II); phyllo-aetio porphyrin was therefore (I). Hence the two ethyl groups are in position 2 and 4, and the one in position 7 is produced by decarboxylation of propionic acid residue.



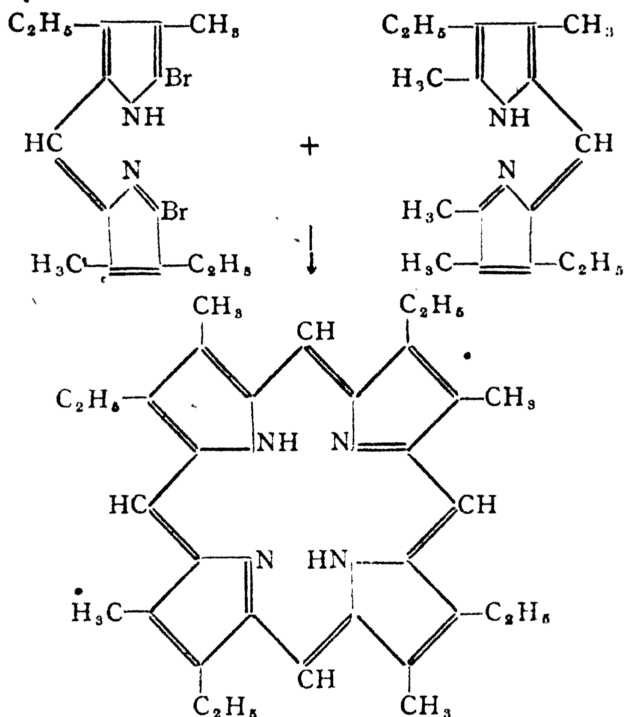
phyllo-aetioporphyrin I



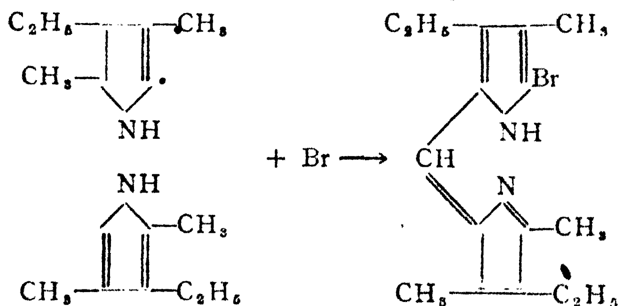
pyrro-aetioporphyrin II

A number of methods have been developed by H. Fischer for the synthesis of aetioporphyrin.

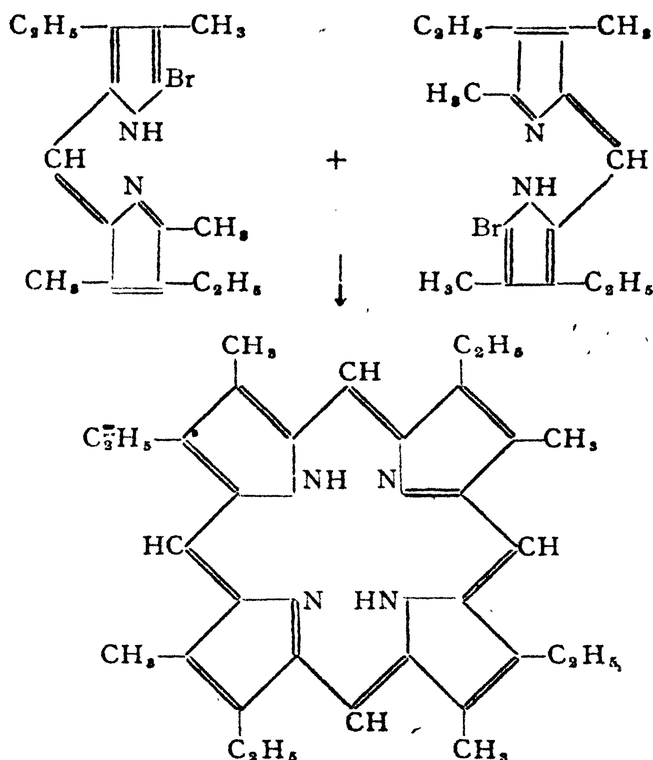
(1) α-α′ Di-bromo derivatives of dipyrryl-methenes are made to interact either with α-α′-dimethyl or bromo-methyl-derivatives of dipyrryl-methenes in the presence of succinic acid :—



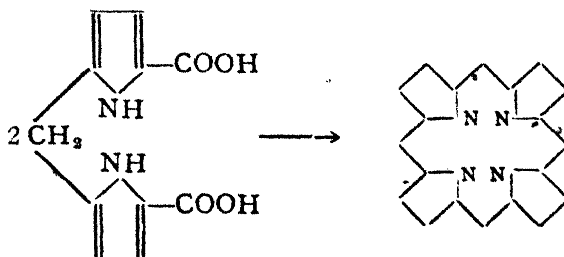
(2) Krypto-pyrrole is first condensed with bromine to from a methene derivative :



The methene derivative, on treatment with concentrated sulphuric acid or formic acid, or succinic acid at 180–190° gives aetioporphyrin:

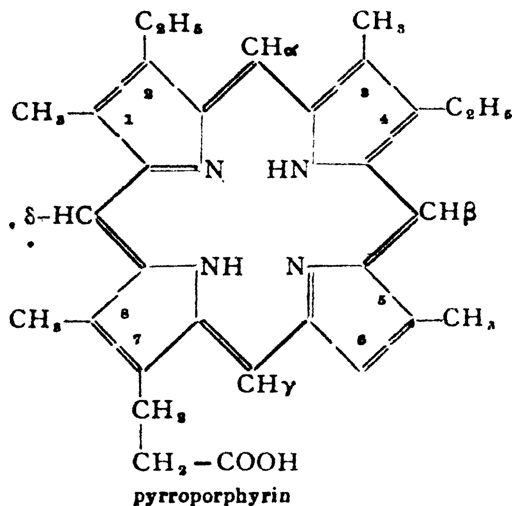


(3) Another synthesis consists in heating α-α′ di-carboxylated di-pyrryl methanes in presence of formic acid:

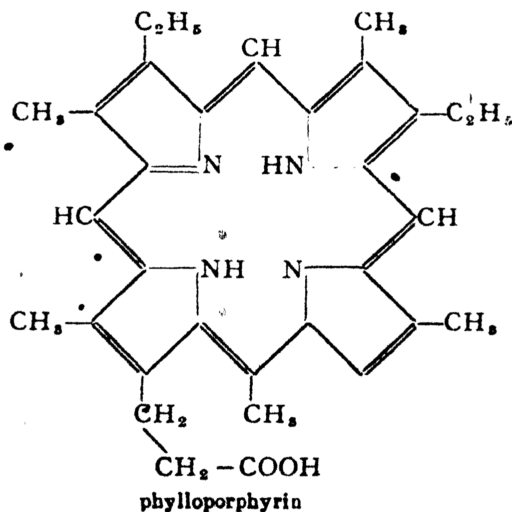


The above syntheses have been used by Hans Fischer to obtain all the four isomeric aetioporphyrins, which differ in the relative positions of the methyl and ethyl substituents.

Similarly, the following porphyrins have been synthesised and their constitutions established :



pyrroporphyrin

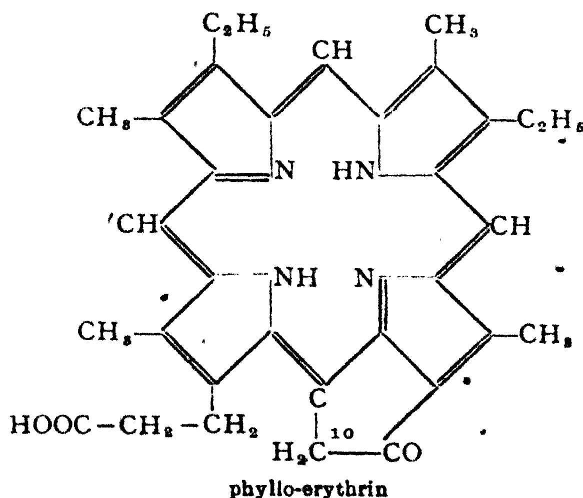


phylloporphyrin

Phylloporphyrin is thus, γ-methyl-pyrroporphyrin ; rhodoporphyrin is 6-carboxylic pyrroporphyrin.

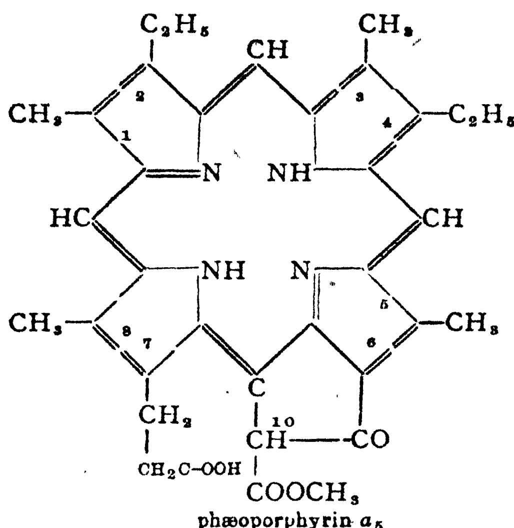
Relation of Chlorophyll a to phyllo-erythrin :—Phaeophorbide *a*, on treatment with HI in glacial acetic acid at 50 to 60°, gives phaeoporphyrin a_5 $C_{35}H_{36}O_5N_4$. It is isomeric with phaeophorbide and contains a—COOH and a—$COOCH_3$ group ; on further treatment with HI, the carbomethoxy group is eliminated and phyllo-erythrin $C_{33}H_{34}O_3N_4$ is formed ; the latter is also obtained directly from phaeophorbide *a* by refluxing it with 20% HCl. Spectroscopically, phylloerythrin was shown to be a porphyrin. It contains a reactive CO— group, which can be reduced to CH_2 by the Wolff-Kishner method. It also forms an oxime and lastly, the presence of 'carbocyclic carbonyl' is revealed by spectro scopic evidence.

The structure of phyllo-erythrin was then elucidated both by analytical methods and by synthesis by Fischer.



phyllo-erythrin

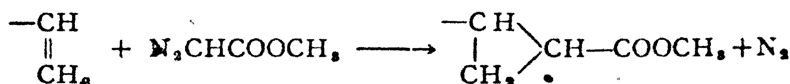
Phaeoporphyrin a_5 would then be derived by placing a —$COOCH_3$ group on C_{10}, as such a group would be readily eliminated being in α position to CO.



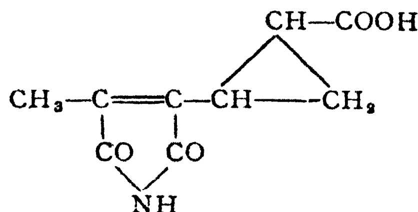
phæoporphyrin a_5

Now phaeoporphyrin a_5 is isomeric with phaeophorbide and is formed by the action of HI in glacial acetic acid. It is a case of isomerisation which involves the migration of two H atoms somewhere in the molecule to the vinyl group to form the C_2H_5 group. The presence of the vinyl group has been established as follows :

Methyl phaeophorbide was treated with methyl diazo-acetate, when the vinyl double bond is attacked and a cyclopropane derivative is formed.

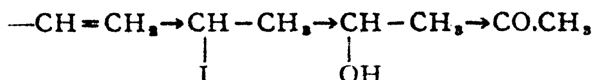


The latter on vigorous oxidation gives rise to methyl-maleic-imide-cyclopropyl-carboxylic acid :



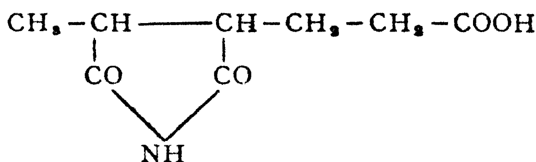
The exact position of the vinyl group is indicated by the following evidence :

Oxy–phaeorporphyrin a_5, obtained by the oxidation of phaeophorbide in glacial acetic acid medium and (HI) is found to contain two CO groups, as it forms a dioxime. Phaeophorbide contained only one *CO* group. The formation of the other is explained as follows :—



This is known as the "oxo reaction." The acetyl compound thus obtained is identical with "oxo–phyllo–erythrin. The latter is synthesised from 2–des–ethyl phyllo–erythrin, by acetalysing it with CH_3COCl in presence of $SnCl_4$. Hence the $-CO-CH_3$ must be position 2; the vinyl group is therefore in position 2.

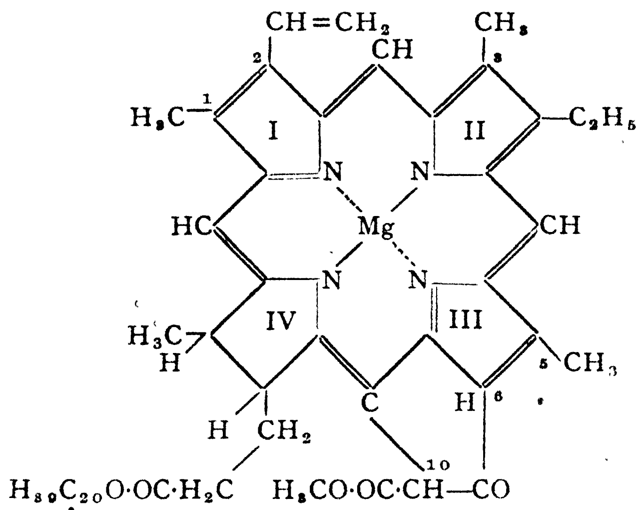
The positions of the two H atoms which migrate to form the C_2H_5 group in position 2 are now indicated to be 7 and 8. Phaeoporbide on oxidation gives an acid fraction from which dihydro haemitinic acid has been isolated.



This imide can come from the nucleus IV i. e. the positions 7 & 8. The position of the phytyl group is determined by the pyrolysis of phaeophorbide. The latter carries a $-COOH$ group (which must have carried the phytyl group) and a $-COOCH_3$ group. The COOH survives pyrolysis and this is characteristic of a $-CH_2-CH_2-COOH$ group. Hence the propionic acid residue must have carried the phytyl group.

Mode of linking of the Mg atom : The latter is not linked through oxygen atoms as aetio phyllin $C_{31}H_{34}N_4Mg$ still carries the Mg atom though all the oxygen atoms are eliminated. It is possibly

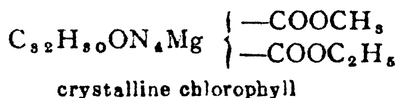
linked through N atoms, because removal of Mg atoms gives porphyrins which are more basic (N – Mg→NH.) Hence the structure for chlorophyll is :—



Chlorophyll *b* differs from chlorophyll *a* in possessing a formyl group (—*CHO*) in place of the methyl group in position 3.

The above formula for chlorophyll *a* thus contains four pyrrole nuclei which are slightly different. Conant and his collaborators determined the basicity of chlorophyll derivatives by potentiometric titration method and compared them with the values obtained for pyrrole derivatives of known and definite constitution. They concluded that chlorophyll contains : one pyrrole nucleus (II), one iso-pyrrole nucleus (III), one pyrrolinine nucleus (I) and one dihydro-pyrrole nucleus (IV). Thus the experimental evidence is in good agreement with the formula that has been provisionally assigned. There are other modifications of the above structure in which the single and double bonds form completely conjugated systems. They represent the different resonance forms. In the given formula, the carbocyclic ring (attached to pyrrole nucleus III) is less stable on enolisation which is in good agreement with the experimental facts. In the alternate formulas, the carbocyclic ring becomes more stabilised by the presence of two double bonds, on enolisation.

CRYSTALLINE CHLOROPHYLL :— This was first isolated by Borodine when he used alcohol for the extraction of the pigment from the leaves. The phytol content of this preparation is very low and Willstatter has advanced the following explanation. The green leaves of the plant contain along with the pigment an enzyme, *chlorophyllase.* Chlorophyll *a* or *b* which is a diester suffers alcoholysis, under the influence of this enzyme. The phytyl radical $C_{20}H_{39}$ is exchanged for the ethyl C_2H_5 one. Thus, crystalline chlorophyll is an ethylchlorophyllide.

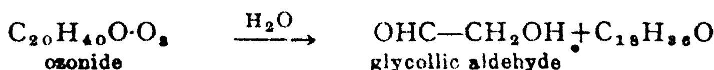


crystalline chlorophyll

Phytol :— It is obtained from chlorophyll (*a* or *b*). It is a thick oil, boiling at 145° (0.03 mm.). Its composition is $C_{20}H_{40}O$. Its structural formula is based on the investigations of Willstatter, Fischer and Lowenberg.

ANALYTICAL EVIDENCE :—The inspection of the molecular formula indicates the presence of a double bond. This is confirmed by the results of ozonolysis and oxidation.

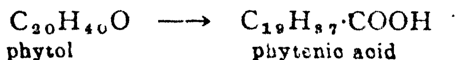
(*a*) On ozonolysis, phytol gives glycollic aldehyde



As the splitting takes place at the position of the double bond, the formula for phytol may be written as :—



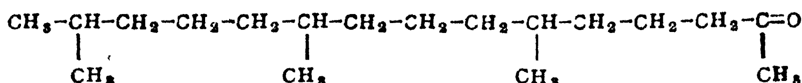
(*b*) The results of chromic acid oxidation further confirm the presence of a primary alcoholic group, because on such oxidation phytol gives phytenic acid :—



i. e., a mono-basic acid is formed which contains the same number of carbon atoms as the original molecule.

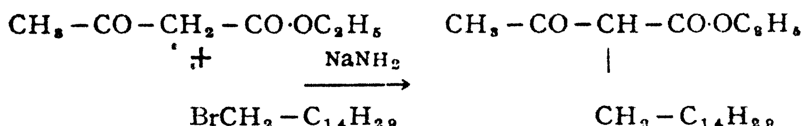
The nature of the carbon framework is revealed from the constitution of the ketone $C_{18}H_{36}O$ formed on ozonolysis. The latter is

shown to be a saturated methyl-ketone and on the assumption that phytol may be built up of reduced isoprene units, it was suggested that the ketone was:

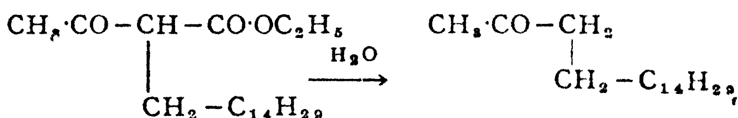


and this structure was subsequently proved by its synthesis from hexadydro farnesol.

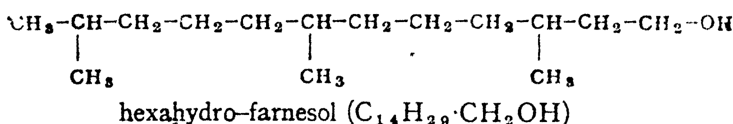
Hexahydro–farnesyl–bromide is made to interact with aceto-acetic–ester to form the corresponding hexahydro–farnesyl derivative of aceto–acetic ester:—



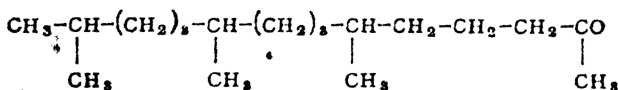
On ketonic hydrolysis, the ketone $C_{14}H_{29}CH_2CH_2{\cdot}COCH_3$ is obtained which is identical with the ketone obtained from phytol



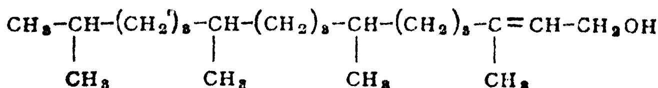
Now the structure of hexahydro–farnesol is known which is:



Hence, the structure of the ketone $C_{18}H_{36}O$ is:

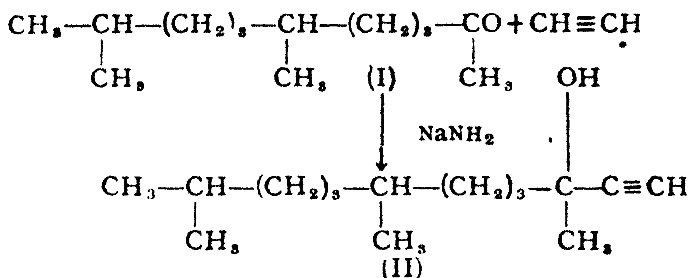


The structure of phytol is:—

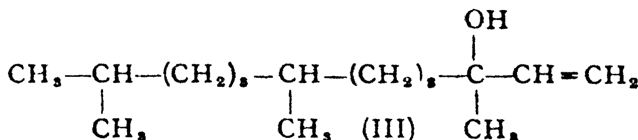


SYNTHESIS OF PHYTOL:—The above formula has been confirmed by a synthesis by Fischer and Lowenberg. The starting point

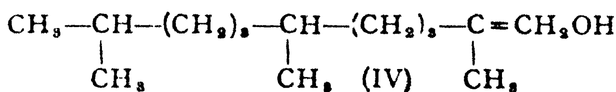
is hexahydro-pseudo-ionone (I), readily obtained by catalytic hydrogenation of ($Pd/CaCO_3$ and H_2) of ionone, available from citral and acetone. It is condensed with acetylene in presence of sodamide to form (II) :—



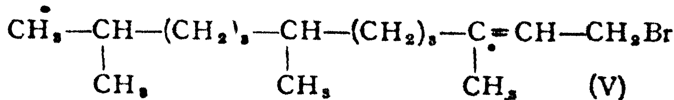
On reduction with sodium and moist ether or with $Pd/CaCO_3$ and H_2 the acetylene linkage is reduced to ethylenic one to give (III) :—



Acetic anhydride converts (III) into an isomeric primary alcohol (IV) (an acetyl derivative is first formed which is hydrolysed to give the alcohol)



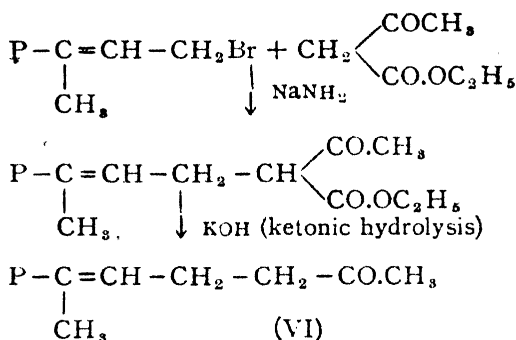
With phosphorus bromide, the compound (IV) is changed into its bromide (V) :—



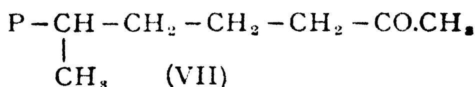
(writing P for $(CH_3-\underset{CH_3}{\underset{|}{CH}}-(CH_2)_3-\underset{CH_3}{\underset{|}{CH}}-(CH_2)_3-)$, we

have (V) as $P-\underset{CH_3}{\underset{|}{C}}=CH-CH_2Br$. This is condensed with aceto-

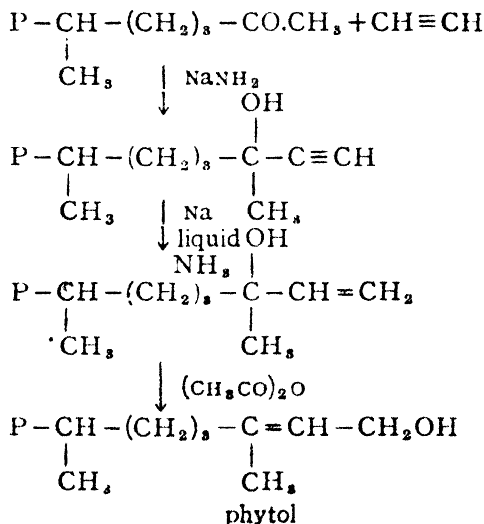
acetic-ester in presence of sodamide to form (VI):—



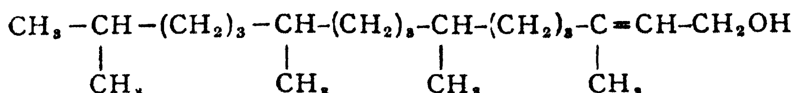
The compound (VI) is hydrogenated with H_2 and $Pd|CaCO_3$ to form (VII):—



This ketone (VII) is now condensed with acetylene in presence of sodamide. The acetylide derivative so formed is reduced to an ethylenic one; on treatment with acetic anhydride, an isomeric rearrangement occurs and phytol is formed:—



Therefore, phytol is :



$$CH_3-CH-(CH_2)_3-CH-(CH_2)_3-CH-(CH_2)_3-C=CH-CH_2OH$$ (with CH_3 branches on each CH and on C)

Colouring Matter of Blood

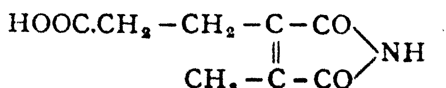
The red pigment of the common blood is called haemoglobin. It is made up of two parts, a protein portion called globin and the non-protein matter called haem. In the living organism, haemoglobin is readily changed into oxy-haemoglobin by the absorption of oxygen. It is a loose combination with oxygen which readily gives oxygen again. Thus, it acts as the carrier of oxygen. This constitutes the important function of haemoglobin in the life process. On the other hand, meta-haemoglobin is formed from haemoglobin and oxygen outside the organism. Meta-haemoglobin, on decomposition with $(CH_3CO)_2O$, gives globin and haematin. Haematin and haem are closely related to each other.

Haematin—It has been assigned the formula: $C_{34}H_{32}O_4N_4Fe(OH)$. On treatment with acids, haematin gives salts called haemins; hydrochloric acid, thus, gives the chloride $C_{34}H_{32}O_4N_4FeCl$ known as haemin.

Haematin shows a remarkable resemblance to chlorophyll. Both contain metallic atoms which display an abnormal behaviour. They are stable towards alkalies, but are readily eliminated by acids. Haematin and haem, thus, give the same iron-free compound haemato-porphyrin, $C_{34}H_{36}O_6N_4$. As in the case of chlorophyll (*a* or *b*) the final degradation product is an aetioporphyrin $C_{32}H_{38}N_4$.

The formation of common decomposition products which are closely related to one another, in the case of the two natural products, suggests that they must possess the same basic structure. The extensive researches involving both analytical and synthetic work of H. Fischer and his school have culminated in the elucidation of the structure of haemato-porphyrin and haemin.

ANALYTICAL EVIDENCE :—(*a*) Oxidation of haemato-porphyrin with chromic acid, gives haematic acid :—



$$\begin{array}{l} HOOC.CH_2-CH_2-C-CO \\ \qquad\qquad\qquad\quad \| \qquad\quad \rangle NH \\ \qquad\qquad\quad CH_3-C-CO \end{array}$$

which indicates the presence of pyrrole nucleus. (*cf.* oxidation of phyllo-porphyrin).

Other oxidative degradation products that have been isolated are: methyl-ethyl-maleic imide and carboxyhaematic acid :—

```
                            COOH
                           /
CH3—C=====C—CH2—CH
    |     |        \
    |     |         COOH
  O=C     C=O
     \   /
      NH
```

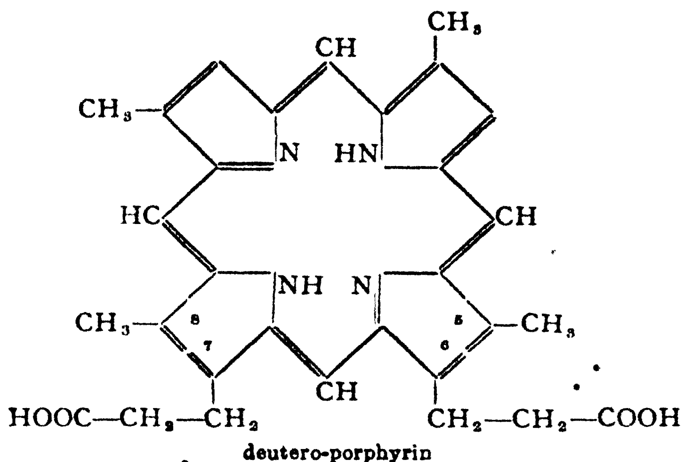
(*b*) On reduction with hydriodic acid and acetic acid, the product was a mixture of bases, from which haemo-pyrrole, krypto-pyrrole, and phyllo-pyrrole etc. have been isolated. At the same time, the acids derived by replacing the ethyl group of the bases by propionic acid residues, have also been obtained. Lastly, haemato-porphyrin was converted into mesoporphyrin, which can finally be changed into aetio-porphyrin $C_{32}H_{38}N_4$.

SYNTHETICAL EVIDENCE :—Now, aetio-porphyrin has been built up of four pyrrole nuclei, and H. Fischer has carried out outstanding researches which have ended in the synthesis of all the four possible aetio-porphyrins differing in the relative positions of the methyl and ethyl groups.

The aetio-porphyrin from the pigment of blood has been assigned the structure:—

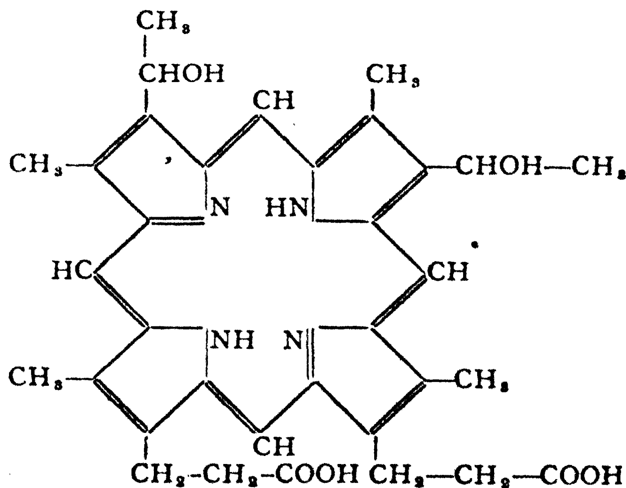
```
          C2H5               CH3
           |        CH        |
          / \\     // \      // \
  CH3—  //    \  //    \   //    \ —C2H5
       \       N          HN     //
        \\____//          |____//
        /                        \
      HC                          CH
       \\                        //
         \_____ NH      N ______/
         /              ||      \
  CH3— //        \    //  \      \ —CH3
       \\        //\\ //    \   //
         \      /    CH       \//
          |                    |
         C2H5                C2H5
```

Starting from deutero-porphyrin, haemato-porphyrin and haemin were synthesised by H. Fischer :—

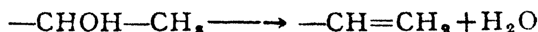


deutero-porphyrin

On treatment with acetic anhydride and stannic chloride, a diacetyl-deutero-porphyrin is obtained, the hydrogen atoms in positions 2 and 4 being replaced by $CO-CH_3$ groups: reduction with boiling alcoholic potash, converts the $CO-CH_3$ groups into $CHOH-CH_3$ groups, and haemato-porphyrin is formed. It has the structural formula :—

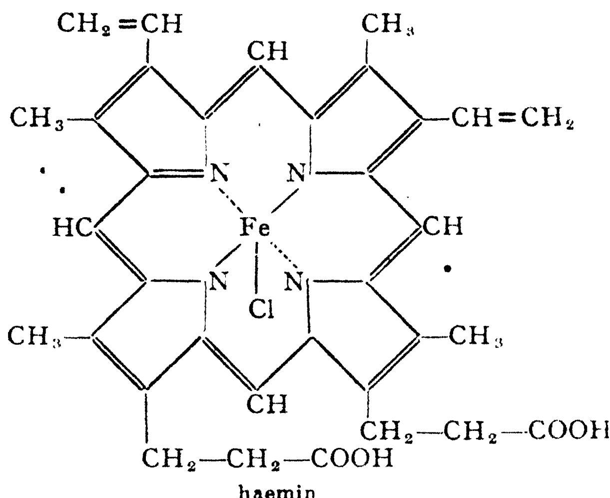


Haemato-porphyrin on heating at 105° in high vacuum, suffers dehydration and gives proto-porphyrin; the vinyl groups are produced in positions 2 and 4 as follows :—



$$—CHOH—CH_3 \longrightarrow —CH{=}CH_2 + H_2O$$

The proto-porphyrin is then changed into haemin by heating it in acetic acid with ferrous acetate with a small quantity of NaCl and a drop of con. HCl



haemin

The synthetic product is identical with natural haemin.

Relation between chlorophyll and haemin :—Chlorophyll plays an essential role in the vital economy of plants. It is, in its presence, that the most vital process, *i.e.*, the assimilation of carbon and nitrogen by the plants takes place. Thus, it has been regarded as the most fundamental agent in the processes elaborated by the plants. Haemin or the constituent of the colouring matter of blood plays a parallel function. It is absolutely necessary for the vital process of the animal. It acts as the carrier of oxygen without which, life itself would be impossible.

Structurally there is a great resemblance between the two. Both contain metallic atoms linked up in a complex manner; chlorophyll contains *magnesium* while haemin contains *iron*. Further, they

appear to be derived from the same fundamental unit, aetio-porphyrin as both of them can be ultimately degraded to similar aetio-porphyrins. Chlorophyll is a diester, haemin is a salt. Thus, we find that the two compounds are related to each other both *functionally* and *structurally*.

Anthoxanthins and Flavones

The yellow colour of many flowers, roots, leaves etc., in nature is due to the presence of various pigments. They can be divided into two classes : *(a)* carotenoids and *(b)* anthoxanthins.

The anthoxanthins, comprise an important group of yellow pigments, that occur widely distributed in plants. They are the glycosides of flavones or flavonols in combination with glucose or rhamnose. They are found closely associated with the *anthocyanins*, the blue or red natural pigments of flowers and berries, and sometimes with the tannins. On hydrolysis, they give the sugar free pigments called anthoxanthidins or flavones; the latter have been used as dyestuffs; in fact, many of the older yellow mordant dyes, used before the development of the modern synthetic dyestuffs, were the anthoxanthidins *i.e.* flavone pigments. Some of the important among such dyestuffs are weld, quercitron bark, the old and young fustic and Indian Piuri or yellow. Luteolin from the weld was the oldest European natural dyestuff. The anthoxanthins also occur in the form of colourless glycosides in the corollas of several white flowers. The white flowers when placed in ammonia vapour, turn yellow owing to the presence of flavones.

General Properties : These yellow pigments are crystalline compounds soluble in water and alcohol. Ferric chloride gives a dull-green or brown colouration. They are precipitated from their alcoholic solutions by lead acetate. They readily dissolve both in dilute acids and alkalies. They are, thus, amphoteric in behaviour. The solution in acids is due to the basic properties of the oxygen atom of the γ-pyrone unit present in their molecules. These oxonium salts are more highly coloured than the original pigments from which they are derived, but are readily hydrolysed by water. They, thus, differ from the anthocyanidins which form relatively stable oxonium salts.

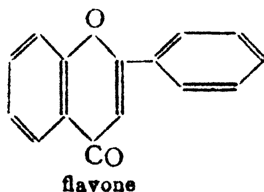
Classification :—The structural chemistry of these yellow pigments has been elucidated through the extensive researches of A. Kostanecki, Herzig and A. C. Perkin. The fundamental structural unit of these natural pigments is the γ-pyrone unit. Hence a very systematic classification based on structural units has been possible. According to this classification, the natural pigments are divided into the following classes :—

(i) the flavones and flavonols which are derived from 2-phenyl-benz-γ-pyrone, flavonones or dihydric flavones and the isomeric chalkones also occur in nature as pigments.

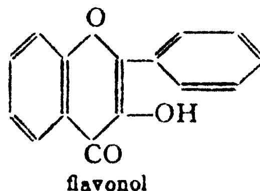
(ii) the isoflavone pigments, which are derived from 3-phenyl-benz-γ-pyrone, and

(iii) the xanthones which are derived from dibenzo-γ-pyrone.

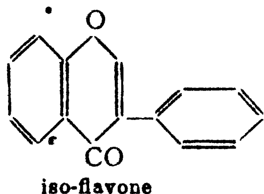
The fundamental structural units mentioned above are represented as follows :—



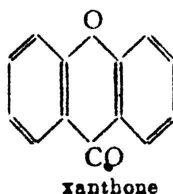
flavone



flavonol



iso-flavone



xanthone

The natural pigments are the hydroxy-derivatives of these structural units. They thus possess phenolic properties and are mordant dyes. The chromophoric group of these flavone derivatives s—*CO—CH*=*CH*—; the chromophoric power is greatly affected by the orientation of *OH* groups ; *OH* groups in position 3′ and 4′ produce a deep-yellow shade, while in positions 5 and 7, they exert only a minor effect. A hydroxyl group in position 3, produces a pale-yellow, but it enhances the effect of hydroxyl groups in positions 3′ and 4′, so that an orange compound is obtained.

Some of the typical pigments belonging to each class are :—

(a) FLAVONES :

Flavone : 2-phenyl-benzo-γ-pyrone,
Chrysin : 5-7-dihydroxy-flavone,
Apigenin : 5-7-4′-trihydroxy-flavone,
Luteolin : 5-7-3′-4′-tetra-hydroxy-flavone.

(b) FLAVONOLS :

Galangin : 5-7dihydroxy-flavonol,
Fisetin : 7-3′-4′-trihydroxy-flavonol,
Kampferol : 5-7-4′-trihydroxy-flavonol,
Quercetin : 5-7-3′-4′-tetra-hydroxy-flavonol,
Myrecetin : 5-7-3′-4′-5′-penta-hydroxy-flavonol.

(c) XANTHONES :

Euxanthone : 1-7-dihydroxy-xanthone,
Gentisein : 1-3-7-trihydroxy-xanthone,
Gentisin : 1-7-hydroxy-methoxy-xanthone.

(d) ISO-FLAVONES :

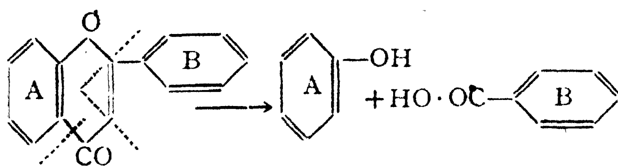
Gentisein : 5-7-4′-trihydroxy-iso-flavone,
Tectorigenin : 5-7-4′-trihydroxy-6-methyl-iso-flavone,
Irigenin : 5-7-3′-trihydroxy-6-4′-5′-trimethoxy-iso-flavone.

Flavones and Flavonols

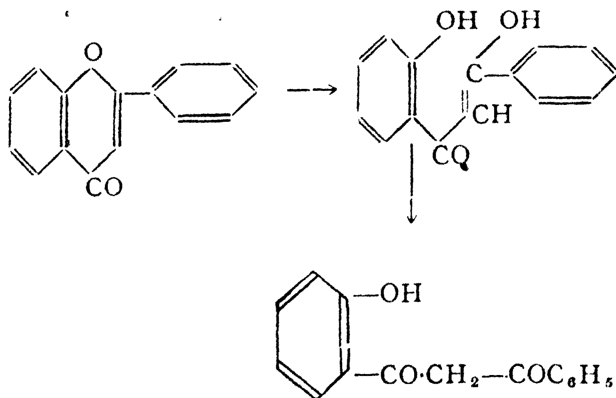
METHODS OF ISOLATION :—As they occur chiefly as glycosides, the plant material is extracted with boiling water: the tannins are removed as Pb salts by the addition of Pb acetate. The extract is then diluted with water, acidified with *HCl* and heated to boiling for some hours ; the glycoside is completely hydrolysed and the colouring matter flavone or flavonol is precipitated. It is extracted with alcohol and may be further purified by acetylation and fractional crystallisation of the acetate. It may also be purified by recrystallisation from suitable solvents like benzene, carbon disulphide, alcohol etc. (The anthoxanthins are not isolated).

Determination of the structure of the flavones. The structure of the natural flavone pigments has been established by degradation and synthetic methods. The natural pigments, anthoxanthins are glycosides. They are hydrolysed to the sugar-free pigments, anthoxanthidins or flavones or flavonols. The structure of the latter has been elucidated by alkaline degradation under different conditions.

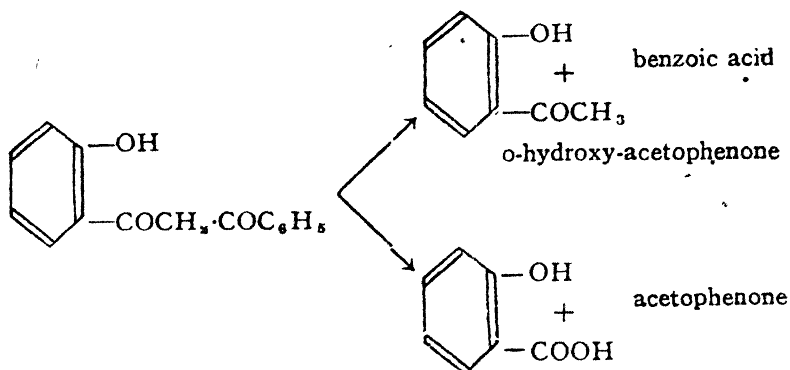
(i) By fusion with alkali like potassium hydroxide; the pyrone nucleus is disintegrated with the formation of *(a)* a phenol and *(b)* an aromatic acid. Thus, with flavone, we have :—



(ii) By boiling with a concentrated solution of alkali, the degradation is less drastic and takes place in stages.

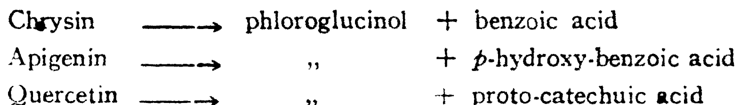


The latter is further degraded by "acid" and "ketone" hydrolysis, so that four different products of cleavage are obtained.



(*iii*) By aspirating air through a dilute alkaline solution of the sugar-free pigment : the products of disintegration are a phenol and a hydroxy acid ; quercetin gives phloroglucinol and proto-catechuic acid. Usually, the flavones are not readily decomposed by this method ; the flavonols, however are rapidly attacked.

The decomposition products of a few typical flavones and flavonols by one of the above methods are :—



Chrysin ⟶ phloroglucinol + benzoic acid
Apigenin ⟶ ,, + *p*-hydroxy-benzoic acid
Quercetin ⟶ ,, + proto-catechuic acid

Thus, the phenol obtained from all of them (which are the most common flavones) is the same i. e. *phloroglucinol*. The aromatic acid obtained, however, is different in all the cases. The difference between them is therefore, conditioned by the number of the hydroxyl groups present in the *phenyl* radical. The structure of these decomposition products, therefore, reveals the structure of the original pigments.

N.B.—Phloroglucinol has all the *OH* groups in *meta* positions to one another ; one of these *OH* groups comes from the γ-pyrone oxygen atom ; the hydroxy-benzoic acid results from the phenyl nucleus.

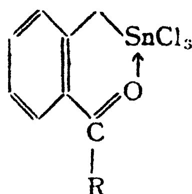
The position of the Sugar residue. The natural pigments occur as glycosides. The position of the sugar molecule is established by first methylating the pigment and subsequently hydrolysing the

methylated pigment with alkali. A hydroxyl group is now generated owing to the opening up of the pyrone ring and also owing to the hydrolysis of the sugar molecule. The presence of *free* OH groups (except the *one* coming from the pyrone ring) will indicate the position originally occupied by the sugar residue. As a result of a large amount of research work, it has been found that generally, position 3 in the case of flavonol is occupied by the sugar molecule; position 5 and 7 are also found to carry sugar molecules.

Disposition of the OH groups in the flavone and flavonol molecules :—Many reactions have been developed for the diagnosis of the positions of the hydroxyl groups in the flavone or flavonol molecules.

(i) A hydroxy group in position 5, shows resistance to methylation by CH_2N_2.

(ii) Flavones with *OH* in position 5, give the Pfeiffer reaction: with $SnCl_4$ in C_6H_6 a substitution product of the type :—



is obtained; if the *OH* is in any other position, a double compound $HO\text{-}C_6H_4\text{-}CO\text{-}R\text{-}SnCl_4$ is formed.

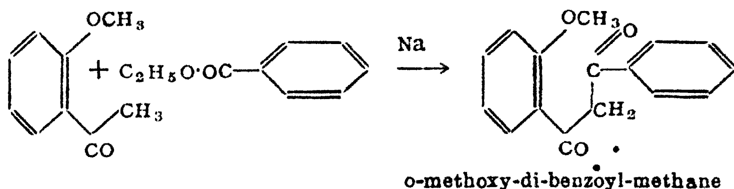
(iii) Flavones containing *OH* groups in position 5, 6 and 7 give the Bargilleni's test: on reduction with *Na*-amalgam and alcohol, green flocks are observed.

(iv) Flavones containing *OH* groups in *ortho* or *para* positions to each other react in alkaline solution in the cold, with o-dinitrobenzene to give deep-violet products; *OH* groups in *ortho* positions to each other give reddish-brown colouration with a mixture of acetic acid and ammonium molybdate.

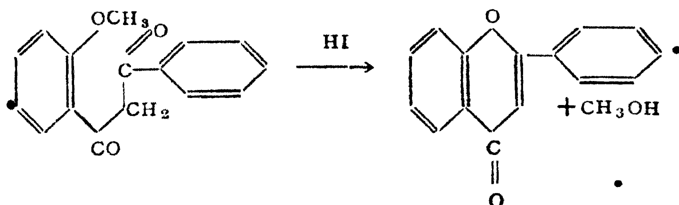
(v) Lastly, as a class, the flavones give yellow coloured Pb-salt with basic lead acetate while flavonols give with the same reagent red coloured salts.

(b) **Synthetic Methods.** The constitutions arrived at, by the above degradation methods have been further confirmed by syntheses. Thus flavones and flavonols are synthesised by (i) Kostenecki's method (ii) Allan–Robinson's aroylation method (iii) Baker-Venkatraman's method and (iv) Chalkone method.

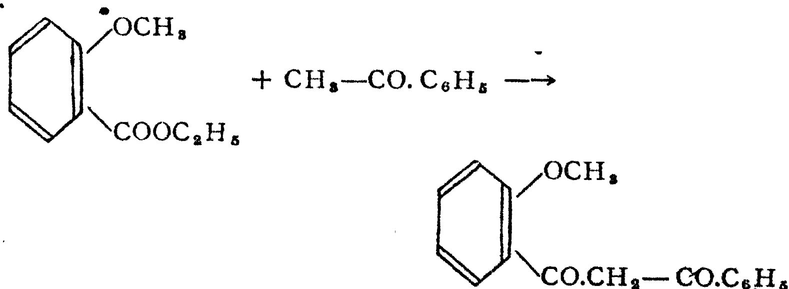
1. *Kostanecki's method from o-hydroxy-acetophenone and benzoic acid* :—o-Methoxy acetophenone is condensed with the ester of the aromatic acid in presence of metallic sodium :—



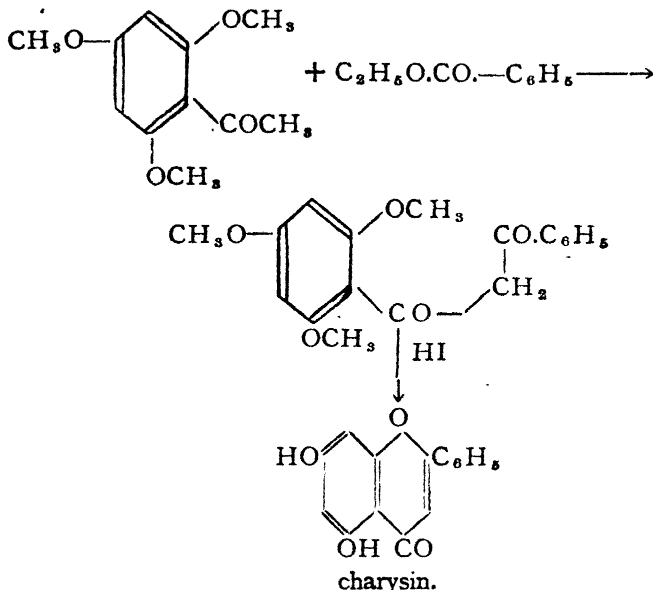
o-Methoxy-di-benzoyl-methane formed, is boiled with hydriodic acid when ring closure takes place and flavone is obtained :—



2. *From acetophenone and o-hydroxy-benzoic acid* in presence of sodium : o-methoxy-benzoic acid ester is condensed with acetophenone to form o-methoxy-dibenzoyl-methane, which is then demethylated to flavone :

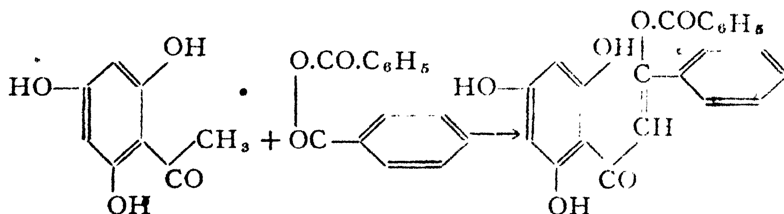


The latter on heating with con. HI gives flavone. In an analogous way, starting from ethylbenzoate and phloro–aceto phenone–trimethyl ether, Kostanecki obtained chrysin.

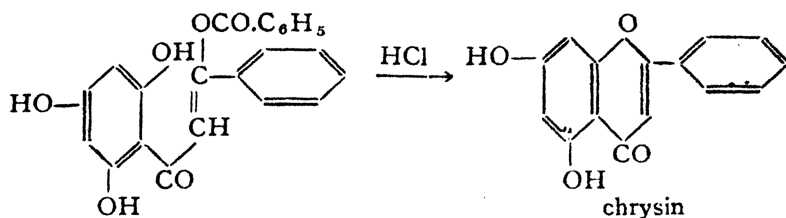


charysin.

3. *Aroylation method* :— This is the Allan-Robinson method capable of very wide application. It consists in heating together a mixture of phloro–acetophenone, the anhydride, and the sodium salt of an aromatic acid. Chrysin—a typical flavone—is thus synthesised by heating together phloro-acetophenone, sodium benzoate and benzoic anhydride at 180-185° for 9 to 10 hours. The probable course of the reaction is :—

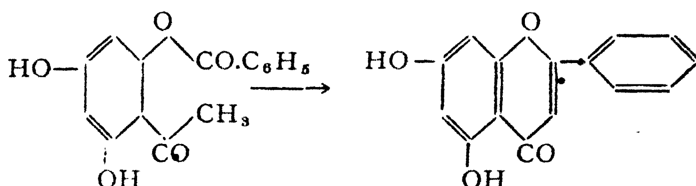


The latter on boiling with hydrochloric acid, loses a molecule of benzoic acid, with the closing up of the pyrone ring and the formation of chrysin.

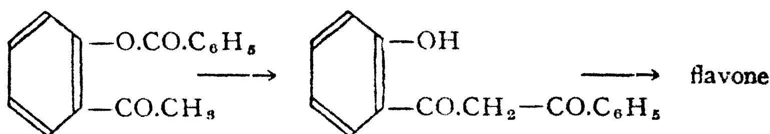


chrysin

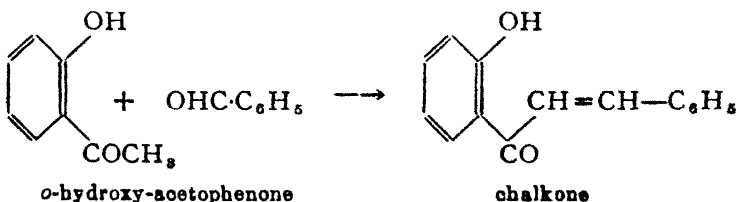
Or, phloro-acetophenone may be benzoylated to give benzoyl-phloro- acetophenone, the latter may then undergo cyclisation to give chrysin.



Baker—Venkatraman's method : In this method 1·3 deketone is obtained by the action of pulverised KOH in pyridine on the o-benzoate of *o*-hydroxy-aceto phenones. The diketone on treatment with con. H_2SO_4 yields the flavone.

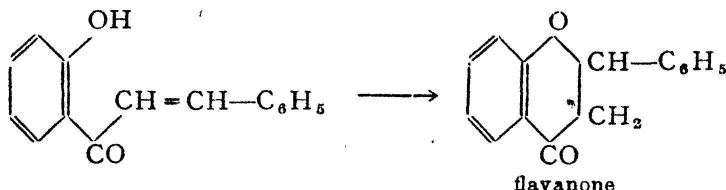


4. *The chalkone method :*—Another general method consists in the conversion of chalkones into flavones. o-Hydroxy-acetophenone is condensed with an aromatic aldehyde to form a chalkone in presence of 10% NaOH. (alcoholic or aqueous).



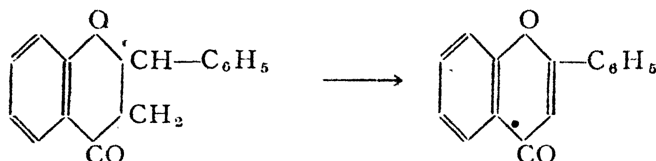
o-hydroxy-acetophenone chalkone

The chalkone is then converted into a flavone in three different ways: in one method, the chalkone is first converted into a flavanone which is dihydroflavone, by boiling with alcoholic alkali or acid (HCl).



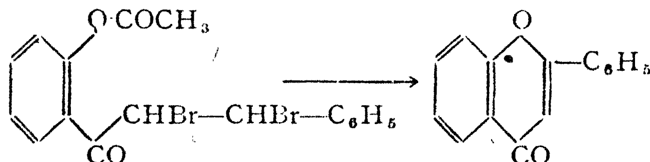
flavanone

The flavanone is then treated with bromine and subsequently with alcoholic potash to give the flavone.



Or the flavanone dissolved in alcohol is boiled with sodium acetate, and iodine is added till a permanent colour is formed; flavanone is dehydrogenated to flavone or still better method is to employ I_2 in glacial acetic acid in presence of K-acetate. A purer form of flavone is formed.

In the second method, the dibromide of the acetylated chalkone is treated with alcoholic KOH, to give the flavone, with the elimination of HBr molecules.



Chrysin may be obtained by this method, by starting from phloro-acetophenone and benzaldehyde.

In the third method, the chalkone is directly converted into flavone, by boiling it with SeO_2 in amyl alcohol.

We shall now discuss the constitution of a typical flavone pigment *e. g.* chrysin.

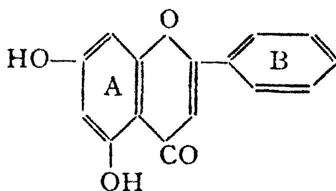
CHRYSIN :—It is present in the poplar buds. It is obtained from the buds of the North American *Populus monilifera balsamifera*, by precipitating an alcoholic extract with lead acetate. It forms yellow plates m. p. 275°. It dyes wool mordanted with aluminium hydroxide a pale-yellow shade. Its constitution is based on the following evidence: *(a)* Its molecular composition is $C_{15}H_{10}O_4$.

(b) It is soluble in aqueous NaOH and forms a diacetyl derivative with acetic anhydride. This indicates the presence of two phenolic hydroxyl groups.

(c) With hydrochloric acid, an oxonium salt is formed which is more deeply coloured, but is rapidly hydrolysed. This suggests the presence of a γ-pyrone ring in the molecule.

(d) On boiling with alcoholic potash, chrysin gives: *(i)* phloroglucinol, *(ii)* acetophenone, *(iii)* benzoic acid and *(iv)* acetic acid.

The above analytical evidence indicates clearly the presence of two phenyl nuclei, a γ-pyrone ring and two hydroxyl groups. Hence the structure may be represented by :

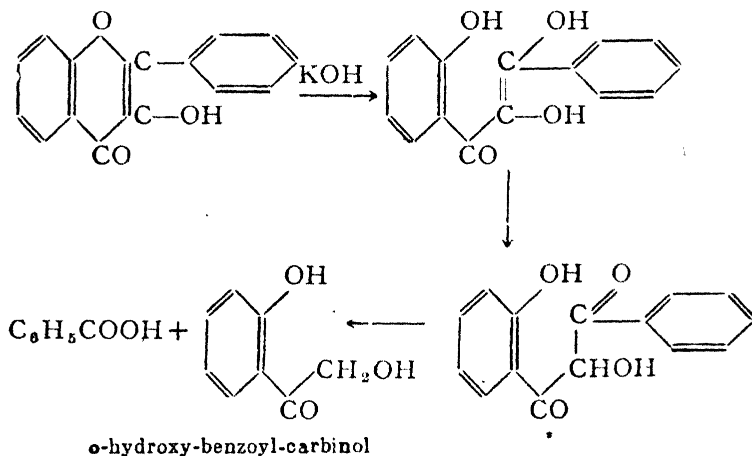


The phloroglucinol molecule is derived from the ring A. (benzo) while benzoic acid comes from the ring B. (phenyl).

Synthesis :—The above structure for chrysin has been confirmed by Kostanecki and independently by Robinson.

Structure of Flavonols. Many naturally occurring yellow pigments are flavonol derivatives *e. g.* quercetin. Their constitution has been established as in the case of the flavones both by degradation and synthetic methods.

(a) Degradation of Flavonol :—On boiling with alcoholic alkali, flavonol is degraded into *o*-hydroxy-benzoyl-carbinol and benzoic acid :—



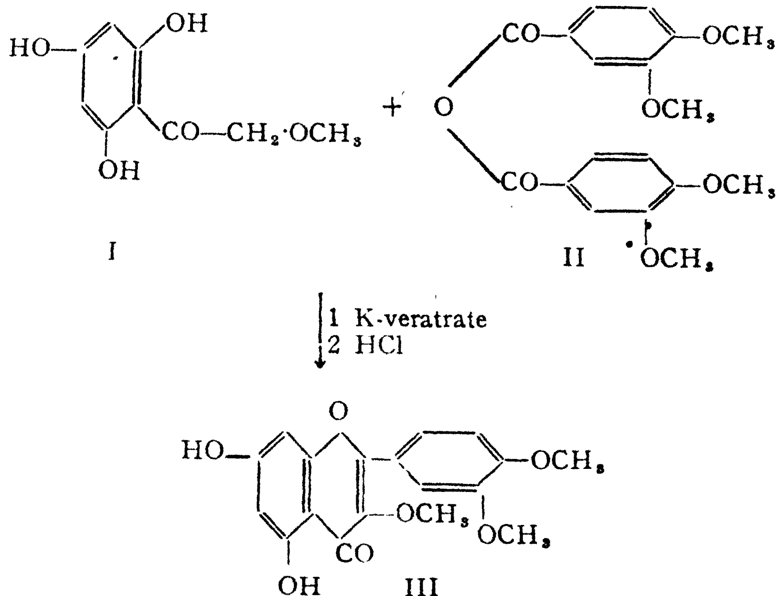
o-hydroxy-benzoyl-carbinol

This reaction is typical of all the flavonol derivatives and has been generally applied to the determination of their structures. Usually, the natural pigments are alkylated (methylated or ethylated) and subsequently boiled with alkali (alcoholic). Fisetin is, thus, ethylated and then decomposed to give 2-hydroxy-4-ω-di-ethoxy-acetophenone and 3-4-di-ethoxy benzoic acid. The structure of the former is further confirmed by its oxidation to 2-hydroxy-4-ethoxy benzoic acid. The free hydroxyl group comes from the opening up of the γ-pyrone ring. In this way, the structure of the original pigment molecule is shown to be built up of two phenyl nuclei linked through a γ-pyrone ring.

(b) Synthesis of flavonols :—There are many methods developed from time to time to synthesise the flavonols; a few typical ones are described below :

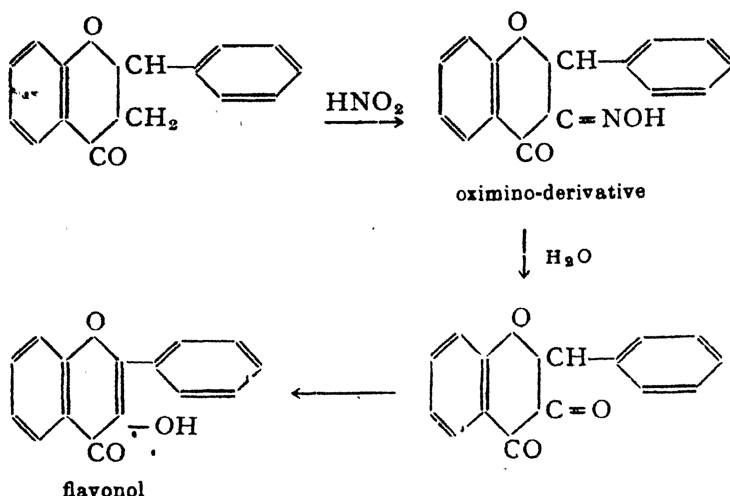
1. Robinson's Method :—Robinson has developed for the synthesis of flavonol derivatives, a method, which is analogous to the one used in the preparation of flavones. ω-Methoxy-acetophenone or its derivative is condensed with the anhydride of an appropriate phenolic acid in presence of its alkali salt. Quercetin, a typical flavonol has, thus, been synthesised. ω-Methoxy-phloro-acetophenone

(I) is condensed with the anhydride of veratric acid (II) in presence of potassium veratrate, by heating at 180 to 200° for several hours. The condensation product on boiling with HCl, loses a molecule of veratric acid and forms the trimethyl ether derivative of quercetin (III).

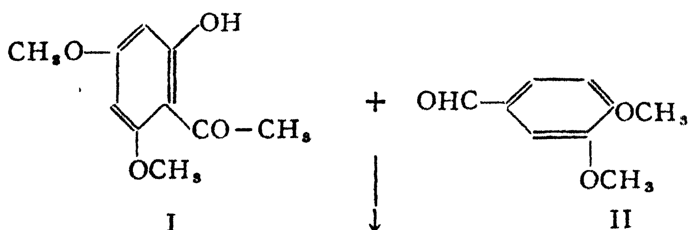


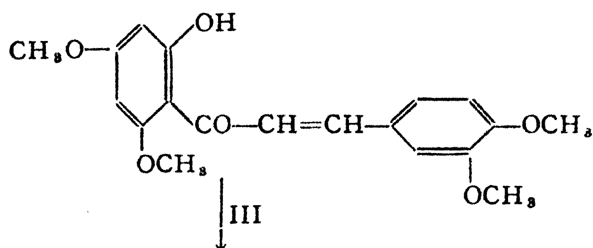
On demethylation with HI, III gives quercetin identical with the natural product. The yield however, is poor. A better method is to use a ω-benzoyl-oxy-derivatives, instead of the methyl ethers; because the benzoyl-oxy-derivatives formed can be much more easily hydrolysed to the flavonols, thus improving the yield.

2. A flavonol derivative may be synthesised from a flavanone in two ways. *(i)* The flavanone in alcoholic solution is treated with nitrous acid (amyl nitrite and concentrated hydrochloric acid) to form an oximino-derivative. The latter, on boiling with 10 per cent sulphuric acid in acetic acid solution, gives flavonol. The oximino group is first hydrolysed to a ketonic group and subsequently the flavonol is formed by an enolic rearrangement.

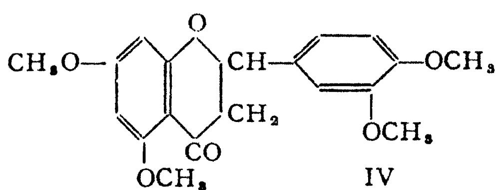


Kostanecki has accomplished a complete synthesis of quercetin by the application of the above method. Dimethyl ether of phloro-acetophenone, (I) was condensed with veratric aldehyde, (II) to form 2-hydroxy-4-6-3′-4′-tetra methoxy-chalkone, (III) in presence of alcoholic alkali. The chalkone on boiling with dilute sulphuric acid (10%) in methanol gives the flavanone derivative (IV). The flavanone, on treatment with nitrous acid (amyl nitrite and hydrochloric acid in methanol) is converted into the oximino derivative (V), which, on hydrolysis, with H_2SO_4 in acetic acid gives the tetramethyl quercetin derivative (VI). The latter, on subsequent demethylation with hydriodic acid, gives quercetin ; schematically we have :

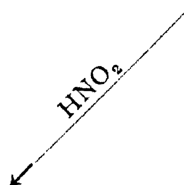




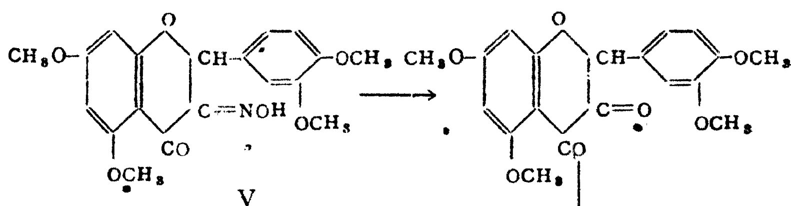
CH₃O—
OH
CO—CH=CH—
—OCH₃
OCH₃
OCH₃
III



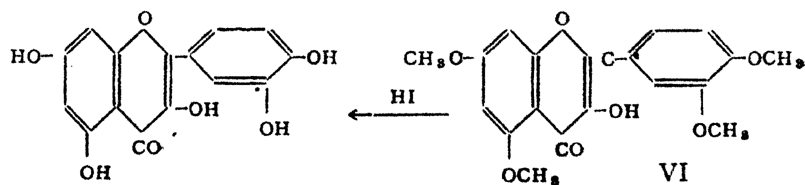
O
CH₃O—
CH—
—OCH₃
CH₂
CO
OCH₃
OCH₃
IV



HNO₂



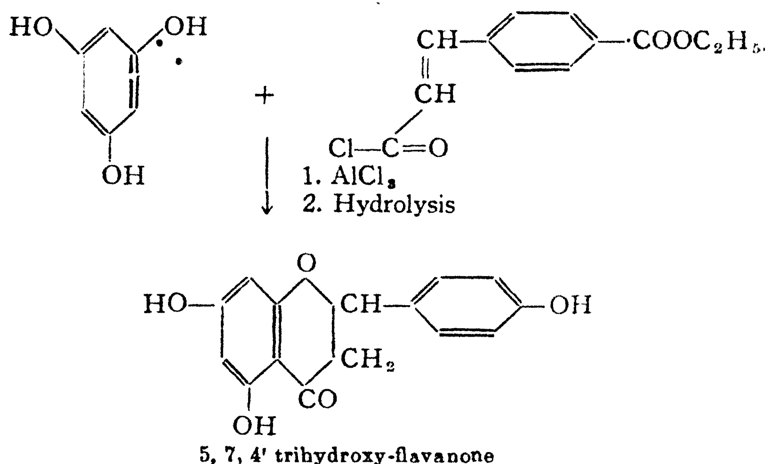
O
CH₃O—
CH—
—OCH₃
C=NOH
OCH₃
CO
OCH₃
V
CH₃O—
CH—
—OCH₃
C=O
OCH₃
CO
OCH₃



O
HO—
—OH
—OH
OH
CO
OH
HI
CH₃O—
C—
—OCH₃
—OH
OCH₃
CO
OCH₃
VI

The synthetic product is identical with the natural product in all its properties. There are two recent modifications; in one of them, the chalkone (III) is directly changed into quercetin-tetramethyl ether by the action of NaOH (16%) and H_2O_2; the tetramethyl ether is then demethylated to quercetin, with HI; In the other procedure, the flavanone (IV) is oxidised with SeO_2 in xylene to tetramethyl ether of quercetin which is demethylated as above.

The flavanone derivatives required in the synthesis of the flavonols are obtained *(i)* from chalkones and *(ii)* by an application of the Friedel-Crafts reaction. A typical example is the synthesis of 5-7-4′ trihydroxy-flavanone.



5, 7, 4′ trihydroxy-flavanone

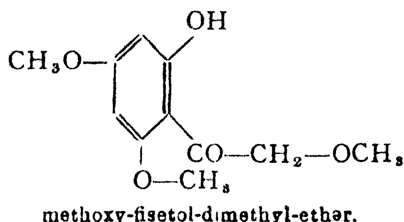
We shall now discuss the constitution of a typical flavonol, quercetin.

QUERCETIN :—It is one of the most widely distributed natural pigments; the more common sources are: the horse-chestnut, the bark of the American oak, tea and red roses. It forms yellow crystals, m.p. 316-17°. Its constitution is based on the following analytical and synthetic evidence.

(a) Its molar composition is $C_{15}H_{10}O_7$.

(b) It gives a penta-acetyl derivative and a penta-methyl ether. This indicates the presence of five hydroxyl groups.

(c) The penta-methyl ether, on boiling with alcoholic KOH, gives veratric acid and methoxy-fisetol-dimethyl ether :

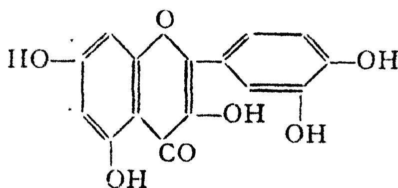


methoxy-fisetol-dimethyl-ether.

(d) Quercetin, on fusion with alkali, gives phloroglucinol and proto-catechuic acid.

(e) It dissolves in con. HCl and gives a deeply coloured solution which indicates the presence of a γ-pyrone unit in the molecule.

The above results clearly suggest the presence of two phenyl nuclei in the molecule, probably linked through a γ-pyrone unit. Of the five hydroxyl groups indicated as under *(b)*, two must be present in each of the two nuclei because, one of the nuclei appears ultimately as phloroglucinol and the other as protocatechuic acid or veratric acid. The remaining hydroxyl group must be present in the γ-pyrone unit and in position 3. The formation of the fisetol derivative mentioned under *(c)* confirms such a conclusion. Hence the structure for quercetin is :

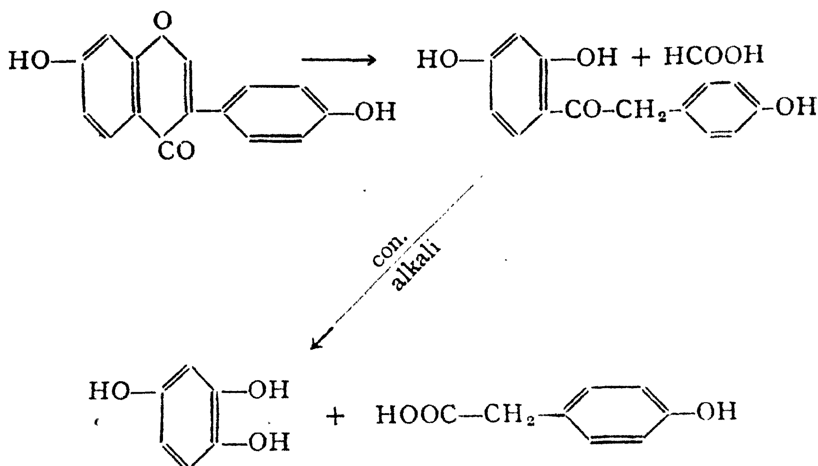


The above structure has been further confirmed by a synthesis.

Isoflavones

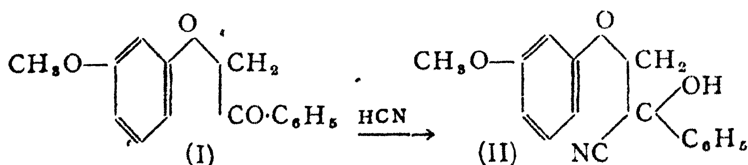
There are some natural yellow dyes which are derived from 3-phenyl–benz–γ–pyrone or iso-flavone. Some typical members are genistein from the flowers and leaves of dyer's broom

and from soya-beans ; and irigenin which is present in the violet ; they both occur as glucosides. A characteristic property of an isoflavone pigment is that on treatment with mild alkali, it is decomposed into an hydroxylated benzyl-*o*-hydroxy-phenyl ketone and a molecule of formic acid. The former is further degraded by concentrated alkali into a polyhydric phenol and hydroxy-phenyl acetic acid.

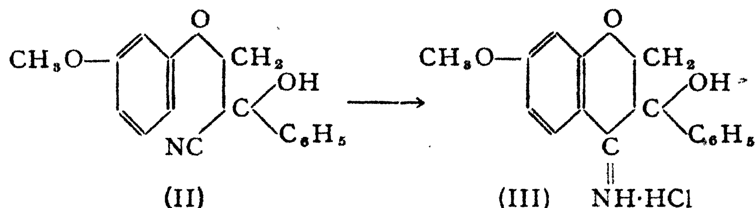


Many methods for the synthesis of iso-flavone derivatives have been developed. The following are the most typical and important ones.

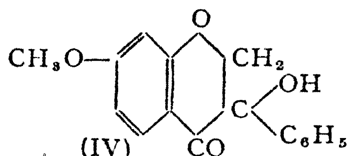
1. Robinson and Baker, employ ω-*m*-methoxy-phenoxy-acetophenone (I) as the starting-point. It is first converted into its cyanohydrin (II) :—



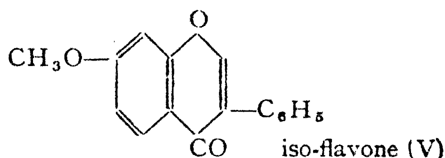
The latter, on treatment with zinc chloride and hydrochloric acid in ether solution, suffers cyclisation to form a ketimine (III).



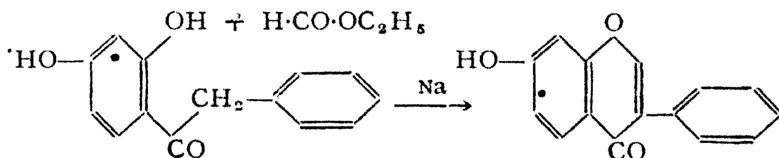
The ketimine derivative is hydrolysed to 3-hydroxy-7-methoxy-iso-flavanone (IV).



With cold concentrated sulphuric acid, dehydration takes place and 7-methoxy-iso-flavone (V) is obtained :—



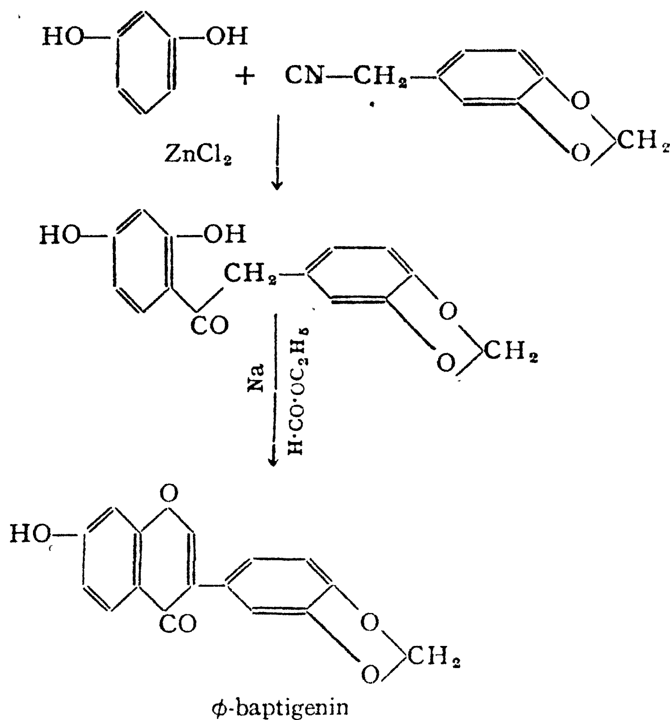
2. More recently, another general method has been developed by Spath and Leder. ω-Phenyl-resaceto-phenone or its derivative is condensed with ethyl-formate in presence of metallic sodium to form an iso-flavone derivative :—



In this way, Spath has synthesised ϕ-baptigenin, 7-hydroxy-3′-4 methylene-dioxy-iso-flavone. The various steps in the synthesis are :—

Resorcinol is condensed with 3-4-methylene-dioxy-phenyl-acetonitrile to give 2-4-dihydroxy-phenyl, 3′-4′-methylene-dioxy-

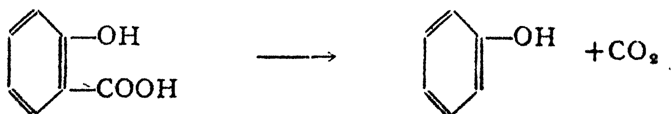
benzyl ketone (Hoesch reaction). The ketone, on condensation with ethyl formate, gives ϕ-baptigenin.

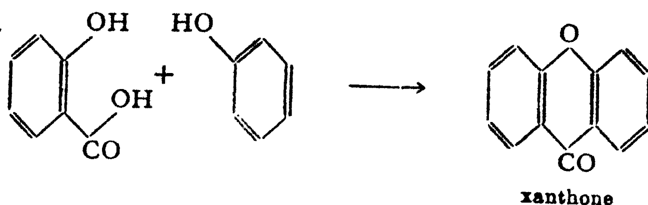


ϕ-baptigenin

Xanthones

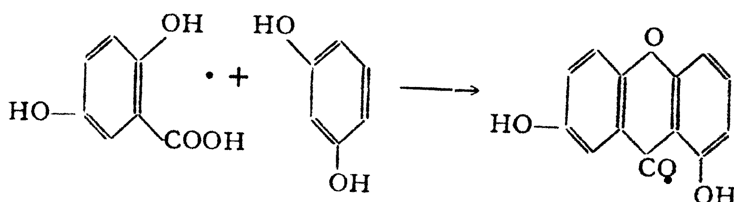
The most common yellow vegetable dyes which belong to this class are euxanthone and gentisin. They are the hydroxy derivatives of xanthone. Xanthone is dibenzo-γ-pyrone. It is related to salicylic acid. The latter, on heating with phosphorus oxychloride or acetic anhydride, gives xanthone. A part of salicylic acid is decarboxylated to give phenol, which then condenses with the remaining salicylic acid to form xanthone :—



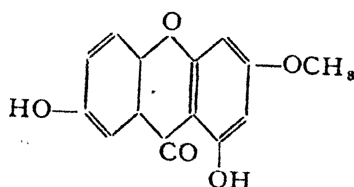


xanthone

Euxanthone is the 1, 7, dihydroxy derivative of xanthone. It is present as the calcium or magnesium salt of euxanthic acid in combination with glucuronic acid, $CHO—(CHOH)_4\text{-}COOH$ as euxanthic acid in Indian yellow. It is obtained from the urine of cows fed on mango leaves. It has also been obtained synthetically when resorcinol is condensed with quinolcarboxylic acid in presence of acetic anhydride :—



The gentian root contains a yellow dyestuff, gentisin which is also a xanthone derivative. It has been assigned the following formula :—



ORIGIN OF COLOUR IN FLAVONES :—The auxochrome group in these pigments is the hydroxyl. The intensity of the colour which these pigments display, depends on the number and position of the hydroxyl groups. The colour is intensified, if two hydroxyl groups are in ortho positions to each other. The natural pigment is a glycoside and one or more hydroxyl groups are involved in glycoside formation. This renders the auxochrome inactive and the flavone glycosides are practically colourless in the plant. However,

the colour of the flavone is developed when the glycoside is hydrolysed and the sugar molecule eliminated. Thus, the white flowers in nature, turn yellow when exposed to the vapours of ammonia as the partial hydrolysis of the glycoside takes place, with the appearance of colour.

Anthocyanins

The anthocyanins or anthocyans are the brilliant *red* and *blue* colouring materials, occurring as glycosides in the cell sap of plants. They are very widely distributed and are found in many flowers, fruits, berries and leaves of plants. The latter owe their brilliant shades of blue and red to the presence of these pigments. They are closely associated with, and related to the yellow pigments—anthoxanthins.

General Properties and Behaviour. These red and blue pigments are soluble in water and other hydroxylic solvents. They, however, do not dissolve in ether, benzene or chloroform. They are amphoteric in nature; with acids, they form the true stable oxonium salts which show extraordinary crystallising properties. Thus, the pigment anthocyanin, on treatment with an acid, like hydrochloric (20%) yields a sugar and an ***anthocyanidin.*** The latter is the ***aglycone*** i.e. the sugar-free pigment. Thus, cyanin a typical anthocyanin from red rose gives on treatment with HCl, cyanidin chloride and glucose.



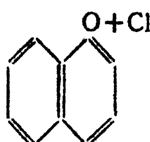
$$C_{27}H_{31}O_{16}Cl \xrightarrow{HCl} C_{15}H_{11}O_{6}Cl + 2C_{6}H_{12}O_{6}$$

The salts of the anthocyanidins with acids are ***red;*** while with alkalies they form salts which are ***blue;*** in neutral medium they are purple.

N.B.—Both anthocyanins and anthocyanidins are crystalline ***chlorides*:** they are referred to as anthocyanin chloride and anthocyanidin chloride or as anthocyanin and anthocyanidin only. The pigments are usually isolated as chlorides although in the plants, they exist in combination with the plant acids.

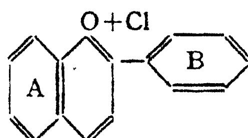
The anthocyanins and the anthocyanidins show strong absorption over the range of 6000 to 2000 A° units, and the absorption spectra of both of them are almost the same.

CLASSIFICATION AND NOMENCLATURE. The structural chemistry of anthocyanins has been systematically investigated and unravelled by the pioneer researches of Willstatter and his school in Germany, and Robinson and his co-workers in England. The fundamental unit of these pigments is found to be a heterocyclic nucleus—the benzo-pyrylium chloride which is an oxonium salt:—



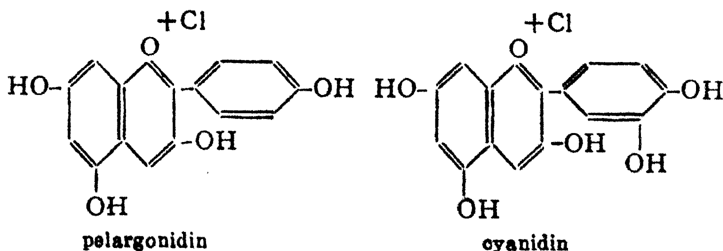
benzo-pyrylium chloride or chromylium chloride

The colouring materials, the anthocyanidins are derived from 2-phenyl-benzo-pyrylium chloride also called flavylium chloride, by replacing

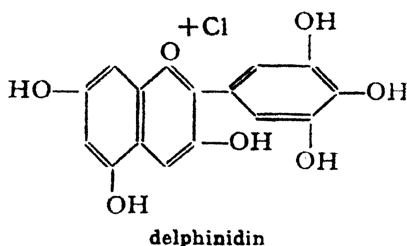


flavylium chloride

the hydrogen atoms of the benzo (A) and the phenyl nucleus (B), by hydroxyl groups or methoxy groups. Many pigments so far isolated from various flowers, berries and leaves of plants are found to belong to three fundamental types, which are the most common. They are: (*i*) pelargonidin, (*ii*) cyanidin and (*iii*) delphinidin types.



pelargonidin cyanidin

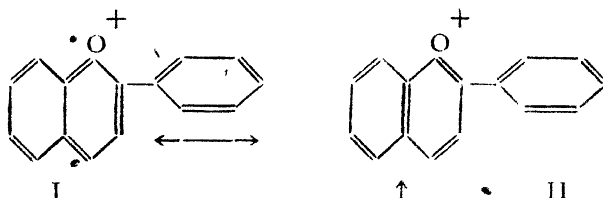


delphinidin

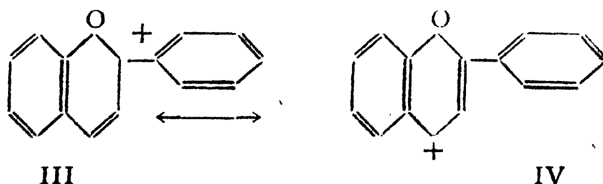
In addition to the above types, there is a fourth one called apigenidin (position 3 carries no hydroxyl group). It is however rare. It is also known as gesneridin chloride. Anthocyanidins are known, which are the methyl ethers of cyanidin and delphinidin types. They are peonidin and malvidin.

According to modern views, the fundamental structural unit is a cation—a resonance hybrid of (*i*) oxonium forms I and II ; (*ii*) of carbonium forms III and IV ; and probably (*iii*) of carbonium forms carrying the positive charge in the benzenoid ring.

(*i*) Oxonium forms :—



(*ii*) Carbonium forms :—



The tendency of oxygen to form an oxonium ion is relatively greater than that of carbon to form a carbonium ion ; hence the forms I and II will make greater contributions to the ground state than the forms III and IV. Further, of the two forms I and II, II is more stable as it contains a naphthalenoid system of linkings while I contains a quinonoid system. Hence formula II has to be perferred to all the others. However, there is some evidence that significant contribution is made by the carbonium form ; because on nitration, of 2-phenyl-benzo-pyrylium derivative, the 2-phenyl group is nitrated at the position 3′ which is meta to the C_1, this indicates that the latter C atom must have carried a fractional positive charge. In this text, the anthocyanidins and the anthocyanins are formulated as oxonium salt of structure II.

The natural pigments—the anthocyanins, are the glycosides derived from one of the above types, by replacement of one or more hydrogen atoms of the hydroxyl groups by a sugar residue. On this basis, they have been classified into :—

(a) 3-mono-glycosides, and *(b)* 3-5-di-glycosides.

The most commonly found sugars are glucose, galactose, rhamnose and some of the pentoses. The most widely distributed anthocyanins are the diglycosides. A few anthocyanins are present as acyl derivatives of organic acids. The following are the acids, so far isolated from such acylated anthocyanins :—

(*i*) malonic acid,
(*ii*) *p*-hydroxy-benzoic acid,
(*iii*) *p*-hydroxy cinnamic acid,
(*vi*) *p*-coumaric acid.

Gentianin chloride ⟶ Delphinidin chloride ⟶ *p*-Coumaric acid.

A few anthocyanins containing amino groups are known but they are very uncommon.

Isolation of the Pigments. The pigment is extracted with methanol or ethyl alcohol containing hydrochloric acid, from the finely ground and dried plant material *(i. e.* the dry petals of flowers*)*, when the soluble chloride of the corresponding anthocyanin is obtained.

It is precipitated from the solution by the addition of ether. It is further purified by redissolving in hydrochloric acid and reprecipitation with ether. Finally, the anthocyanin chloride is recrystallised from alcoholic hydrochloric acid.

Willstatter and his collaborators have used dichlor-picric acid as the reagent for the precipitation of the pigment as the dichorpicrate. The picrate is subsequently changed into the chloride. The use of butyl alcohol has been reported by Rosenheim for the extraction of the pigment from a weakly acid solution.

The anthocyanin is sometimes precipitated as the Pb-salt; the latter is then decomposed by H_2S and the pigment is obtained by extraction with a suitable solvent.

Lastly Karrer has used the chromatographic methods of Tswett for the further separation and identification of the pigments.

DETECTION OF ANTHOCYANINS :—Robinson and his students have developed methods for the qualitative detection of the type of anthocyanin present in a particular plant or flower extract. These methods are based on *(a)* colour reactions with ferric chloride, *(b)* oxidation with dilute alkali, and *(c)* distribution between two immiscible solvents.

FERRIC CHLORIDE TEST :—The extract with amyl alcohol is treated with sodium acetate and a small quantity of ferric chloride. If cyanin is present, the amyl alcoholic solution which is violet in the beginning changes to blue. The test is not given by pelargonidin, peonidin and malvidin.

ALKALI TEST :—A dilute solution of the pigments is shaken up with a small quantity of 10 per cent. sodium hydroxide solution. Oxidation takes place and delphinidin and petunidin are completely destroyed.

DISTRIBUTION TEST :—The natural pigment is distributed between 1 per cent aqueous hydrochloric acid and a mixture of anisole and ethyl iso-amyl ether 5 : 1 which also contains 5 gms. of picric acid per 100 c.c. Pelargonidin, peonidin and malvinidin are readily extracted; cyanidin is extracted to some extent while delphinidin is not taken up at all. Some other immiscible solvents have also been employed.

The most common and typical anthocyanins are cyanin, pelargonin, delphinin, malvin, etc. Cyanin was the first anthocyanin to be isolated; it is the colouring matter both of the red rose and the blue corn flower; in the latter, it is present as the potassium salt.

Determination of the Structure of the Anthocyanins. The structural relationships of a large number of anthocyanins have been elucidated both by degradation methods and by syntheses which are unambiguous.

DEGRADATION METHODS:—The anthocyanin is first boiled with dilute hydrochloric acid to give the anthocyanidin chloride and a sugar molecule. Pelargonin—a typical anthocyanin, thus, with dilute hydrochloric acid gives the anthocyanidin chloride, pelargonidin chloride and glucose:—

Pelargonin + HCl ⟶ Pelargonidin chloride + glucose



$$C_{27}H_{31}O_{15}Cl \qquad C_{15}H_{11}O_{5}Cl + 2C_{6}H_{12}O_{6}$$

The number of OH and OCH_3 groups present in the anthocyanidin are determined by the usual methods.

The anthocyanidin is then decomposed by hot *(KOH)*: the pyrylium ring suffers cleavage, with the formation of a phenol and a phenolic acid.

The results of such a decomposition with the typical anthocyanidins are:—

Pelargonidin ⟶ phloroglucinol + *p*-hydroxy-benzoic acid

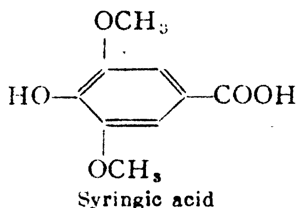
Cyanidin ⟶ phloroglucinol + protocatechuic acid.

Delphinidin ⟶ phloroglucinol + gallic acid.

Thus the phenol obtained in each of these three fundamental anthocyanidins, is the same, namely phloroglucinol. The phenolic acid obtained, however, is different in the three cases. The natural pigments, thus, differ from one another in the number of hydroxyl groups attached to the phenyl nucleus. These differences affect the colour markedly especially in acid medium. Thus, pelargonin is orange red, cyanin is red and delphinin is bluish red.

In the case of anthocyanins which are methyl ethers *e.g.* peonin and malvin, the position of the methoxy groups is indicated by

degradation of the corresponding anthocyanidins with dilute alkali (10 per cent barium or sodium hydroxide). The methoxy groups appear intact in the alkaline decomposition products. Peonidin, thus, gives phloroglucinol and 3-methoxy-4-hydroxy-benzoic acid. These results indicate that the methoxy group in peonidin is in the phenyl nucleus. Karrer has developed another mode of attack which employs hydrogen peroxide (15%) as a reagent and which involves degradative oxidation of the anthocyanin. The latter is treated with hydrogen peroxide when the pyrylium ring opens up between the carbon atoms 2 and 3 : and the methoxy groups remain intact. Thus, the oxidative degradation of malvin chloride gives syringic acid.

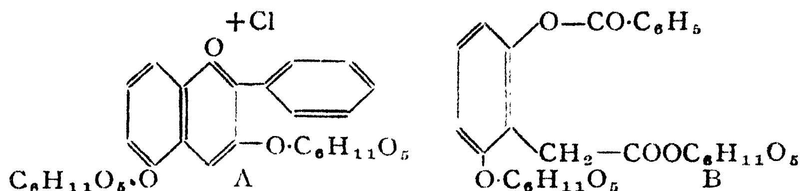


Syringic acid

Malvone is formed as an intermediate compound but its exact structure has not been elucidated. The formation of syringic acid proves that the two methoxy groups are in position 3′ and 5′ (*i.e.* in the phenyl nucleus).

POSITION OF THE SUGAR RESIDUES IN THE ANTHOCYANINS :—There are two methods that have been developed by Karrer to determine the position of the sugar residues in the natural pigment. In one of them, the anthocyanin is methylated and subsequently hydrolysed. The sugar residue is thereby eliminated with the generation of a free hydroxyl group. The identification of the position of the unmethylated hydroxyl group, locates the position of the sugar residue. It is found that a 3-glucoside is rapidly attacked by acid, while 7 and 5 glucosides are hydrolysed with great difficulty. The presence of a free OH group in position 3, makes the molecule susceptible to oxidation with $FeCl_3$; and this reaction is used to ascertain whether a sugar residue occupies this position or not.

The second method involves oxidative degradation of the anthocyanins by hydrogen peroxide in acetic acid (See the detection of the position of methoxy groups in peonidin and malvin above). The pyrylium ring opens up between C_2 and C_3 without removing the sugar residues.

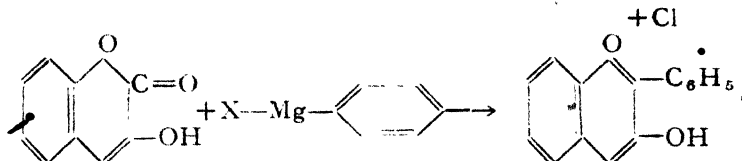


On treatment with dilute NH_3, the compound B, yields one molecule of sugar which was present in position 3 ; the sugar residue in position 5 or 7 is removed only by heating with dilute HCl.

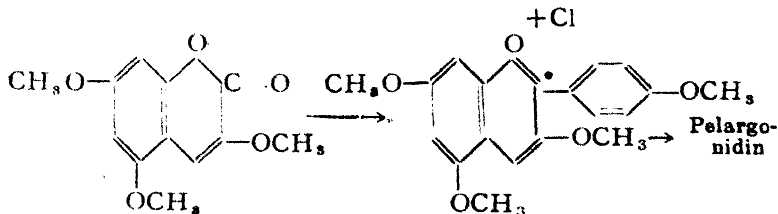
Finally, the results obtained by the above methods are confirmed by the synthesis of the particular anthocyanin by unambiguous methods.

SYNTHETIC METHODS :—Many general methods of synthesising anthocyanidins have been evolved. They are :—

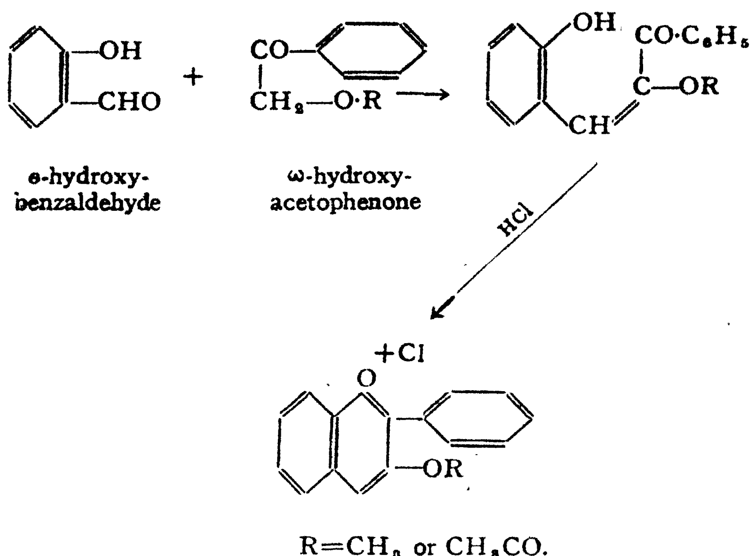
1. A coumarin derivative is converted into an anthocyanidin by the action of a Grignard reagent and subsequent acid hydrolysis :



This method has been employed by Willstatter and his school for the synthesis of the fundamental types of anthocyanidins *e.g.*, pelargonidin and cyanidin. Trimethoxy-coumarin and anisylmagenesium bromide, give a compound which on treatment with HCl and subsequent demethylation gives pelargonidin.



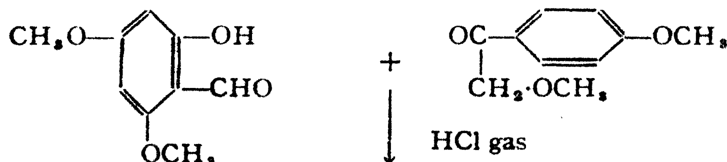
2. *o*-Hydroxy-benzaldehyde or its derivatives are condensed with appropriate derivative of ω-hydroxy-acetophenone, in presence of hydrochloric acid gas in ethylacetate medium.

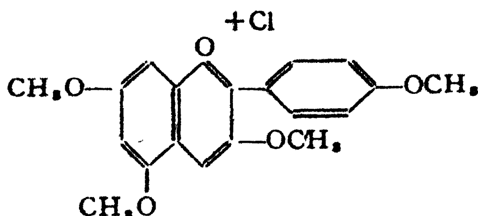


$R=CH_3$ or CH_3CO.

This method was independently and simultaneously discovered by Decker and Fallenberg, and Perkin and Robinson. It is of wide application : a suitable hydroxy-aldehyde is condensed with compounds containing a $COCH_2$–group in presence of *HCl* gas using ether or ethylacetate as the medium. Robinson and his co-workers have synthesised all the fundamental anthocyanidin types by an application and extension of this method. Pelargonidin chloride was first synthesised in the following way :—

2-Hydroxy-4-6-di-methoxy-benzaldehyde was condensed with ω-4-di-methoxy-acetophenone in presence of hydrochloric acid : tetra-methyl-pelargonidin was obtained.





On demethylation, the iodide is first formed, which by treatment with AgCl, is changed into the chloride. The demethylation however has great practical disadvantages, as the removal is too vigorous and non-selective and hence the yield is poor. Robinson avoids these disadvantages by employing the acetyl derivatives, in place of the methyl ethers. Cyanidin chloride is thus synthesised.

3. Anthocyanidins can also be obtained from flavonols by reduction and subsequent treatment with HCl ; the reducing agents used are : (i) Mg and HCl in presence of mercury salts, (ii) Na and alcohol (iii) $LiAlH_4$. This reaction is very easy to carry out and hence is made the basis of a qualitative test for the detection of flavonols.

We shall now discuss the constitution of a few typical anthocyanins.

Cyanin :—This is the most common and typical anthocyanin. It is isolated from the red rose and the blue corn-flower. Its constitution has been established as follows :—

(a) It has the molecular composition $C_{27}H_{31}O_{16}Cl$ and on acid hydrolysis gives cyanidin and glucose



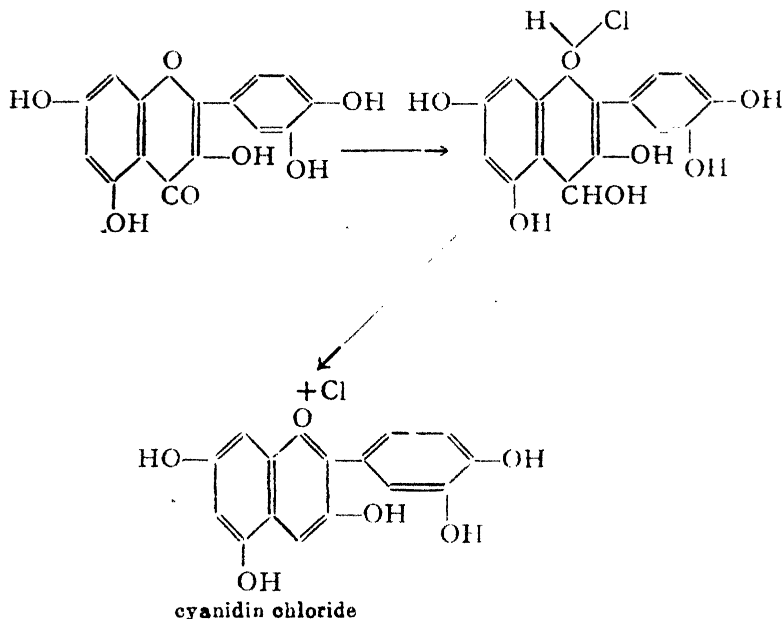
$$C_{27}H_{31}O_{16}Cl \longrightarrow C_{15}H_{11}O_6Cl + 2C_6H_{12}O_6$$

Hence cyanin is a diglucoside of cyanidin ; further the positions occupied by the sugar residues are shown by the usual methods (see p. 510) to be 3 and 5.

(b) Cyanidin forms a crystalline penta-acetyl derivative ; it is also soluble in aqueous NaOH. These results indicate the presence of five phenolic OH groups.

(c) With hot alkali, cyanidin is decomposed into phloroglucinol and proto-catechuic acid. Quercetin also on alkaline degradation gives the same products.

This indicates a close relationship to quercetin, a very common and typical flavonol. This is confirmed as follows : quercetin on reduction with Mg in alcoholic solution containing HCl and Hg, gives a small yield of cyanidin chloride. The reduction of quercetin to cyanidin can be followed schematically :—



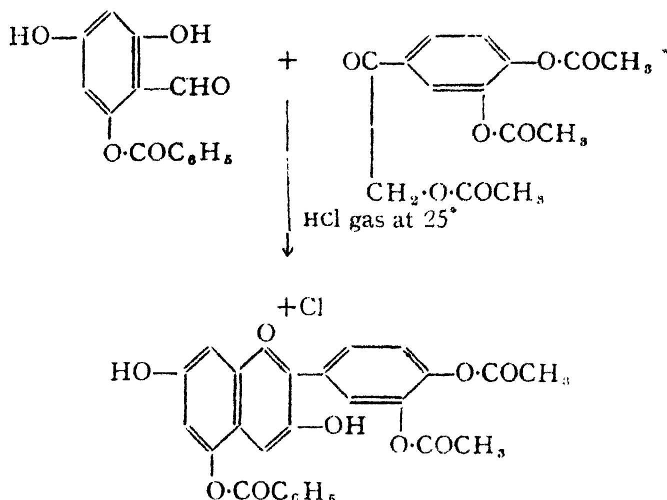
cyanidin chloride

The relatively greater stability of the pigment is due to the presence of the benz-pyrylium system. The above structure also satisfactorily accounts for the existence of 5 hydroxyl groups in the molecule.

Both cyanidin and cyanin have been synthesised and the above structures confirmed. Phloroglucinaldehyde obtained by the Gattermann's reaction from phloroglucinol is benzoylated in position 2.

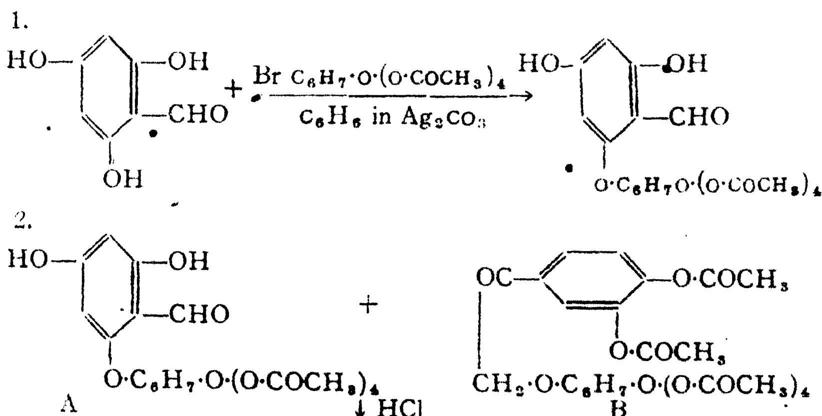
The free aldehyde cannot be used, as it is very reactive and condenses with itself. Mono-benzoylation is found to stabilise the molecule.

The product 2-benzoyl-oxy-4-6-di-hydroxy benzaldehyde is condensed with ω-3-4-tri-acetoxy-acetophenone in presence of hydrochloric acid gas, in ethyl acetate solution, when 5-benzoyl-cyanidin chloride is formed.



The acetoxy groups and the benzoyl group are then removed by hydrolysis with aqueous alcoholic alkali in an atmosphere of H_2, which also opens up the pyrylium ring. On treatment with hydrochloric acid, the pyrylium ring is closed up again with the formation of cyanidin chloride.

Synthesis of cyanin : Cyanin is found to be the 3·5 diglucoside of cyanidin. The synthesis is based on the use of acetobromo-glucose.



$\overset{+}{O}$ Cl

HO— —O·$COCH_3$

O·$COCH_3$

—O·C_6H_7O·$(OCO·CH_3)_4$

O·C_6H_7·O·$(O·COCH_3)_4$

dil. NaOH in atmosphere of H_2
and subsequent acidification

↓

$\overset{+}{O}$ Cl

HO— —OH

OH

—O·$C_6H_{11}O_5$

O·$C_6H_{11}O_5$

cyanin

In a recent modification, the compounds A and B (see above) are converted to A' and B' respectively by the action of Ba $(OH)_2$ and CO_2

HO— —OH

—CHO

O·$C_6H_{11}O_5$

A'

CO— —OH

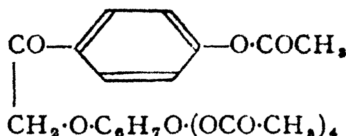
OH

CH_2 – O·$C_6H_{11}O_6$

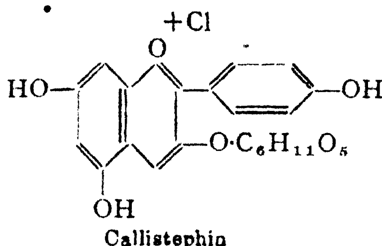
B'

A ' and B ' are carefully dried in vacuo, dissolved in dry ethyl acetate containing $MgClO_4$ and the mixture saturated with HCl gas at O°. Cyanin chloride is formed in good yields.

Robinson and his school have also carried out the synthesis of a few other natural pigments *e.g.* callistephin and malvin. CALLISTEPHIN was the first anthocyanin to be synthesised by Robinson and Robertson. It is a 3-glucoside. ω-Hydroxy-4-acetoxy-acetophenone is condensed with aceto-bromo-glucose in presence of Ag_2CO_3 in C_6H_6 solution to give:

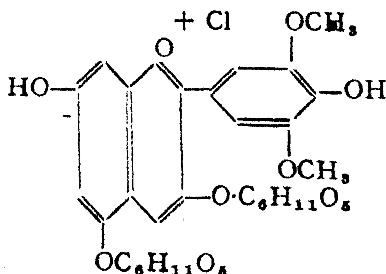


The latter on condensatiou with benzoyl-phloroglucin-aldehyde in presence of *HCl* gas yields the acylated pigment which on hydrolysis with aqueous *NaOH* and subsequent acidification with *HCl* gives the anthocyanin.

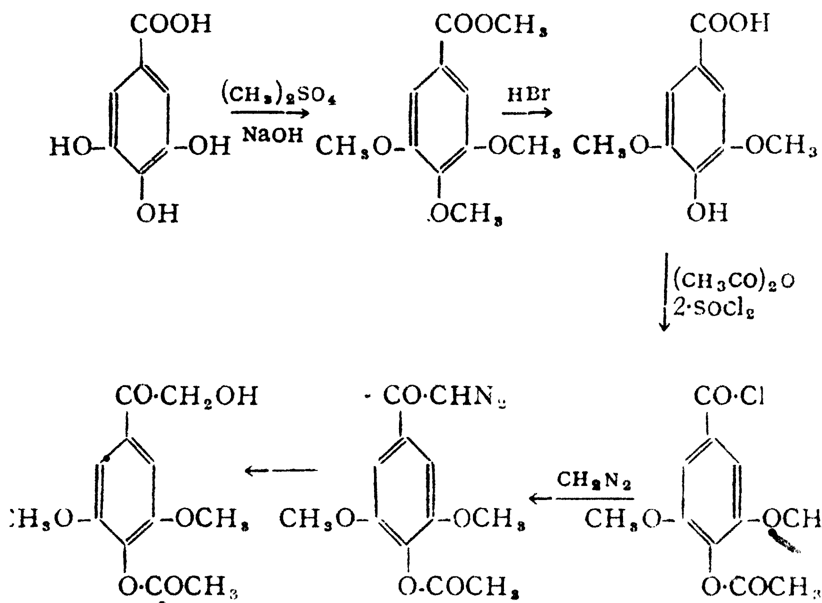


Callistephin

Malvin:—This pigment on hydrolysis with HCl gives malvidin and 2 moles of glucose. Further, it has been shown that it is a 3-5 diglucoside. Lastly, on boiling with alkali, malvidin is decomposed into phloroglucinol and syringic acid. Hence malvin is assigned the structure:

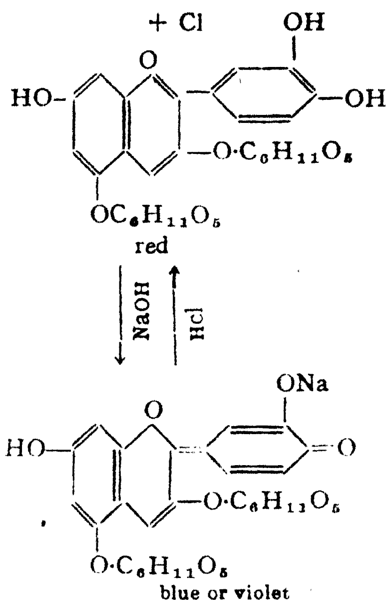


The above structure has been confirmed by a synthesis : tetra acetyl-glucosidyl–phloro-glucin-aldehyde (I) is obtained as mentioned earlier. The ω-hydroxy-4-acetoxy-3, 5-dimethoxy-acetophenone is obtained as follows :



The latter is treated with aceto-bromo-glucose in the usual way to give **II.** **I** and **II** are then condensed in presence of HCl gas in ethylacetate. The rest of the procedure is the same as under the synthesis of cyanin.

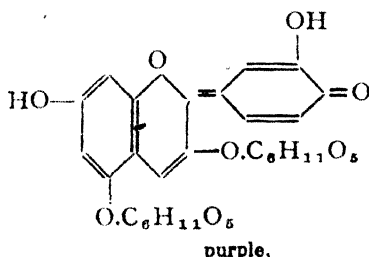
THE COLOUR OF THE ANTHOCYANINS :—The anthocyanins are amphoteric ; with acids, they form the oxonium salts which are coloured red ; when treated with alkali like sodium hydroxide they are converted into metallic salts which are blue or violet. The change in colour is due to alteration in the structure of the molecule. Thus with cyanin,



red

blue or violet

Thus cyanin is red in the pH range 3 or less, which is present in the natural rose ; it is violet at pH 8·5 and is deeply blue at pH 11. The latter conditions exist in the corn-flower and hence it is blue. In neutral condition, the pigment is found to be purple and has been assigned the Structure :



purple,

The colour of the anthocyanin, therefore, changes with alteration in the acidity of the cell sap in the plants. In the presence of the acid the oxonium salt or the pyrylium salt formation is greatly favoured. The alkali, on the other hand, favours the quinonoid structure. Thus, the same molecule assumes two different structure.

forms. Hence, the same pigment can exhibit two different shades of colour and serve as a reddish or bluish colouring material in nature. The same pigment cyanidin accurs both in the red rose and the blue corn-flower. According to Robinson, the main factors that influence the colour and shade of the anthocyanin in the cell sap are :—

(*i*) the type of the anthocyanidin,

(*ii*) the nature of the sugar, and the number of sugar rose dues

(*iii*) the acidity of the cell sap, the acid components, and

(*iv*) the presence of co-pigments like flavones, tannins etc.

It is also found that the greater the number of oxygen atoms, the bluer is the pigment. The affinity of the pigments for mordanted wool also increases with the increase of the hydroxyl groups in the molecule.

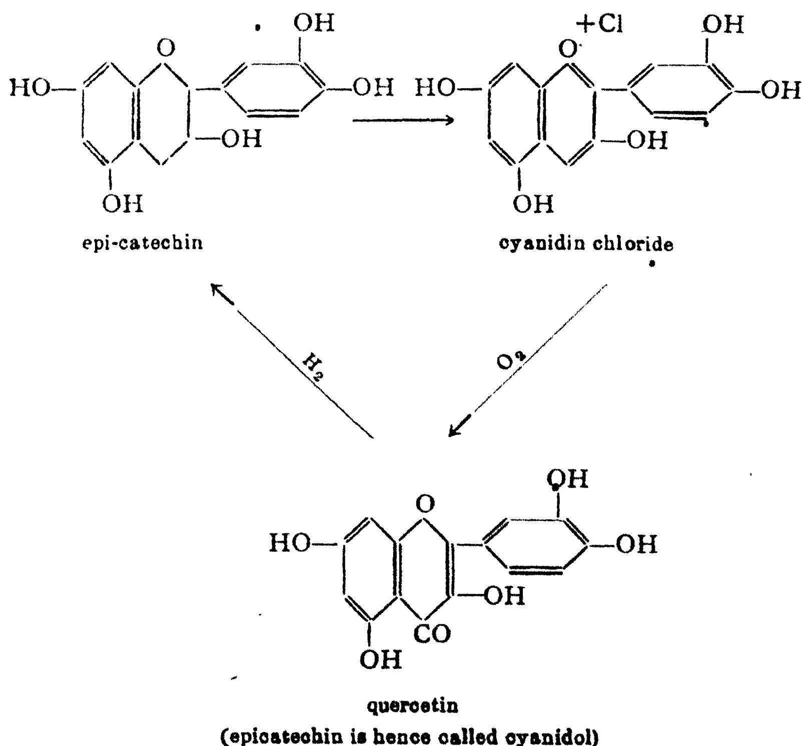
Biogenetic Relationship between Anthocyanidins and Flavones. The anthocyanins, the flavones and flavonol derivatives are often found associated together in nature. The empirical composition of the anthocyanidins, further suggests a simple relationship between them and the flavones. Cyanidin base is obtained by treating cyanidin with aqueoues *Na*-acetate : it is a deep-violet compound with the composition $C_{15}H_{10}O_6$; it is isomeric with luteolin ; similarly delphinidin is isomeric with quercetin. The anthocyanidin and the flavones, on decomposition with alkali, behave in a completely analogous way. Both give a phenol and a hydroxy-aromatic acid. Lastly, both these classes of compounds give oxonium salts. But the salts of the antho cyanins are relatively more stable than those of be anthoxanthidins.

Thus, these two classes of natural pigments are structurally closely related to each other. The flavonol derivatives can be readily converted by reduction into anthocyanidins. Quercetin, on treatment with sodium amalgam and alkali and subsequent reaction with hydrochloric acid, or with magnesium and aqueous alcoholic hydrochloric acid, is changed into cyanidin chloride. Recently, Robinson and collaborators have effected the conversion of flavonols into anthocyanidins by reducing with $LiAlH_4$, and subsequent acidification with HCl. Kaempferol is thus converted into pelargonidin. Quercetion is the most widely distributed flavonol derivative while cyanidin

is the most common anthocyanidin. It thus, appears that the pale-yellow pigments are the progenitors of the most brilliant shades of red and blue in the plant kingdom.

However, the recent investigations of the Robinson school have established the presence of *leuco-anthocyanidins* which are the precursors of the corresponding coloured anthocyanidins. Some of these leuco-compounds, have been isolated in a crystalline form and have been shown to give the coloured pigment, on treatment with hydrochloric acid in presence of oxygen.

Lastly, both these classes of natural pigments are genetically and structurally related to the catechins (*q.v.*). This relationship becomes obvious on an inspection of their respective structural formulæ.



quercetin
(epicatechin is hence called cyanidol)

It has now been possible to effect the reverse conversion of flavylium salts to flavones. Also, flavones or flavonols can be hydrogenated catalytically to catechins. Thus the three classes of compounds probably represent three different stages of oxidation. It is highly probable that the bio-synthesis of these three types of natural products, has a common origin.

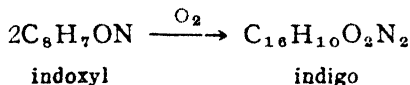
INDIGO

Indigo blue or indigotin is the oldest and one of the most important vat dyes. It is found in the leaves of indigo plants of the species *indigo ferra* and *isatis tinctoria* (wood) in the form of a glucoside indican. The leaves (cut just before flowering) are steeped under water in wooden vessels, at about 50° and the enzyme present (indimulsin) hydrolyses the indican into indoxyl and glucose; the hydrolysis can also be effected by boiling with dilute mineral acids.



$$\underset{\text{indican}}{C_{14}H_{17}O_6N} \xrightarrow{H_2O} \underset{\text{indoxyl}}{C_8H_7ON} + \underset{\text{glucose}}{C_6H_{12}O_6}$$

Some ammonia is formed during enzymatic hydrolysis, which gives with indoxyl a yellowish solution. The latter is run off and aerated by vigorous agitation, when indoxyl (yellow) is oxidised to the blue indigo which is precipitated out. Indoxyl is a yellow compound m. p. 85° and is stable in weakly acid solutions.



$$\underset{\text{indoxyl}}{2C_8H_7ON} \xrightarrow{O_2} \underset{\text{indigo}}{C_{16}H_{10}O_2N_2}$$

The yield of indigo is only 0·2 per cent. The natural indigo thus obtained is a mixture of indigotin (95%), indigo red and indigo gluten.

The indigo of commerce of today is chiefly a synthetic product. The present industrial syntheses are due to Heumann, but it was Baeyer who by his pioneer researches elucidated the constitution of indigo, which rendered synthetic indigo a commercial possibility. The researches of Baeyer extended over a period of about twenty years and are marked with many fruitful reactions and procedures.

We shall, here, briefly narrate the story of the indigo constitution as it was systematically unfolded.

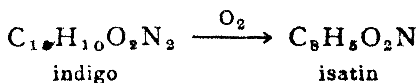
STRUCTURE OF INDIGO:—By 1840, the empirical composition of indigo was established as C_8H_5ON, by Fritsch. It was also known that distillation of indigo with potassium hydroxyde, gave an oil identical with aniline. Some anthranilic acid is also formed. An oxidation product of indigo with nitric acid had been also isolated and was called isatin. But these facts did not go far to give an insight into the architecture of the molecule.

BAEYER'S RESEARCHES:—At this stage, Baeyer took up the problem of the indigo constitution and commenced his systematic and pioneer researches. Indigo $C_{16}H_{10}N_2O_2$ was a highly complex molecule and the complete elucidation of its structure involved the determination of

(i) the simple structural units,
(ii) the constitution of the units,
(iii) the mode of linking of the units in the building up of the complex molecule.

To achieve this, it was necessary to degrade the indigo molecule systematically in a simpler and readily identifiable molecules. Baeyer, therefore, initiated and developed the two fundamental methods:—(a) method of oxidative degradation and (b) method of reductive degradation.

Thus, indigo was subjected to vigorous oxidation with nitric acid, and isatin was the chief oxidation product.



$$\underset{\text{indigo}}{C_{16}H_{10}O_2N_2} \xrightarrow{O_2} \underset{\text{isatin}}{C_8H_5O_2N}$$

Secondly, indigo was distilled at high temperatures with zinc dust when indole C_8H_7N was obtained as the main product of the reaction. These results clearly indicated that indigo, isatin and indole are closely related to one another. Baeyer, then, proceeded to elucidate the structures of isatin and indole molecules. He next established the relationship existing between these three compounds and thus, was able to formulate a structural formula for indigo. He subsequently confirmed it by an elegant and simple synthesis.

At the same time, it was also known that indigo and indoxyl were closely related. Indoxyl on oxidation is converted into indigo. The method of producing indigo from the indigo leaves involves this reaction. Baeyer showed that indoxyl was 3-hydroxy-indole and was isomeric with oxindole. In this way, the inter-relationships existing between indigo and its degradation products were experimentally established.

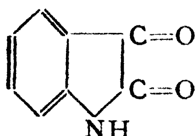
Structure of isatin and indole:—Baeyer established structural formulas for both isatin and indole, based on the following evidence:—

(*a*) Isatin has the molecular composition $C_8H_5O_2N$.

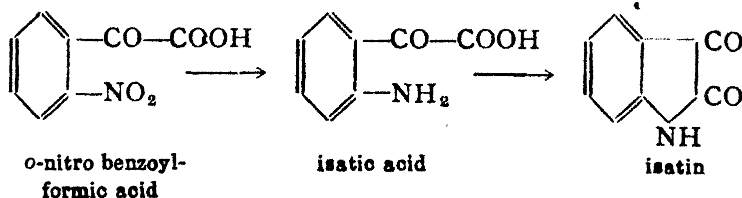
(*b*) It reacts with phosphorus pentachloride to form isatin chloride $C_8H_4ON{\cdot}Cl$. This indicates the presence of hydroxyl group (lactim).

(*c*) It gives an oxime with hydroxylamine, hence a CO group is indicated.

(*d*) On boiling with alkali, it is converted into *o*-aminobenzoylformic acid. Hence the structure for isatin is:



The above structure is confirmed by its synthesis from *o*-nitrobenzoylformic acid by reduction with ferrous sulphate and ammonia. *o*-Nitro benzoyl-formic acid is obtained from *o*-nitro benzoyl-chloride and *KCN*, and subsequent hydrolysis.

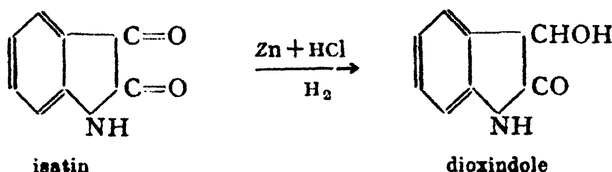


o-nitro benzoyl-formic acid **isatic acid** **isatin**

Isatin was then successively reduced to dioxindole, oxindole and indole. The constitution of each of these compounds was then

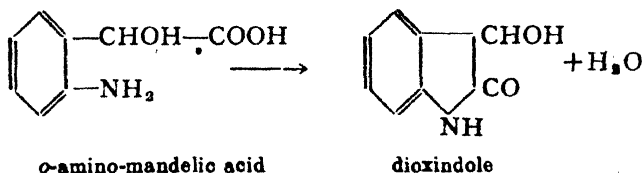
deduced from that of isatin and further confirmed by a direct and unambiguous synthesis.

Isatin with zinc and hydrochloric acid is reduced to dioxindole :—



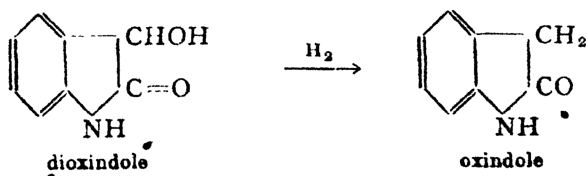
isatin dioxindole

The above structure for dioxindole is confirmed by its synthesis from o-amino-mandelic acid by intra-molecular elimination of water :—



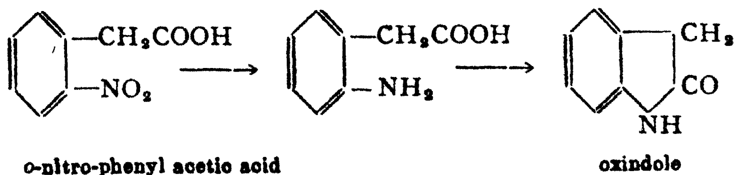
o-amino-mandelic acid dioxindole

Dioxindole, on further reduction with sodium amalgam, forms oxindole :—



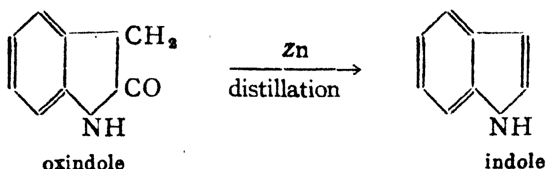
dioxindole oxindole

The latter is formed from *o*-nitro-phenyl acetic acid by reduction, a synthesis which confirms the above structure :—



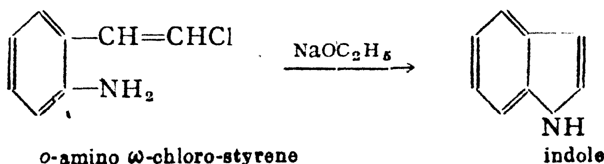
o-nitro-phenyl acetic acid oxindole

Lastly, oxindole on distillation with zinc dust, is converted into indole :—



oxindole indole

This structure for indole is confirmed by its synthesis from *o*-amino-ω-chloro-styrene :—



o-amino ω-chloro-styrene indole

Further, these conversions are reversible.* Thus these results establish the structural formulas for isatin and indole and prove that indole is the fundamental unit present in isatin, dioxindole and oxindole.

Now, indigo can be converted into isatin which can be further converted into indole. Also, indoxyl is obtained from indigo by fusion with potassium hydroxide, and indoxyl is β-hydroxy-indole. Hence, it is obvious that indigo must contain the indole nucleus. An inspection of the compositions of indigo, isatin and indole, reveals that indigo probably contains two indole units.



$C_{16}H_{10}O_2N_2$ indigo



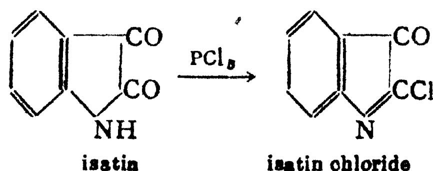
$C_8H_5O_2N$ isatin



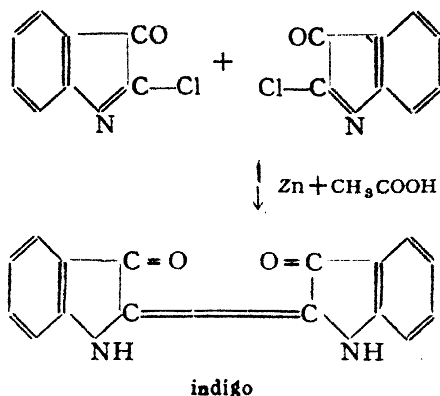
C_8H_7N indole

This relationship is confirmed by the following synthesis :

Isatin with phosphorus pentachloride gives a chloride which, on reduction with zinc and acetic acid and subsequent oxidation forms indigo :



isatin isatin chloride

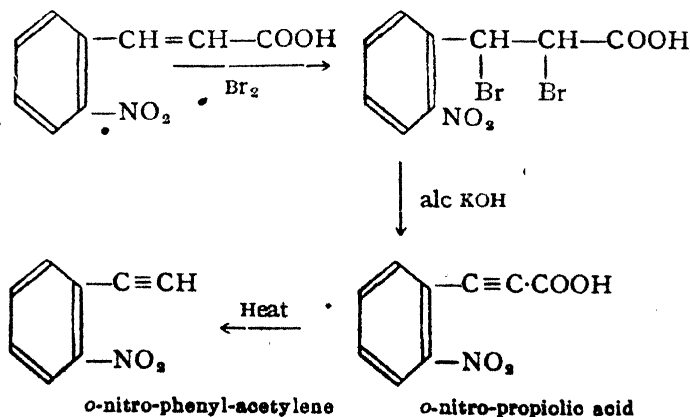


indigo

Probably, isatin chloride is first reduced by Zn and acetic acid, to indoxyl which is then oxidised to indigo.

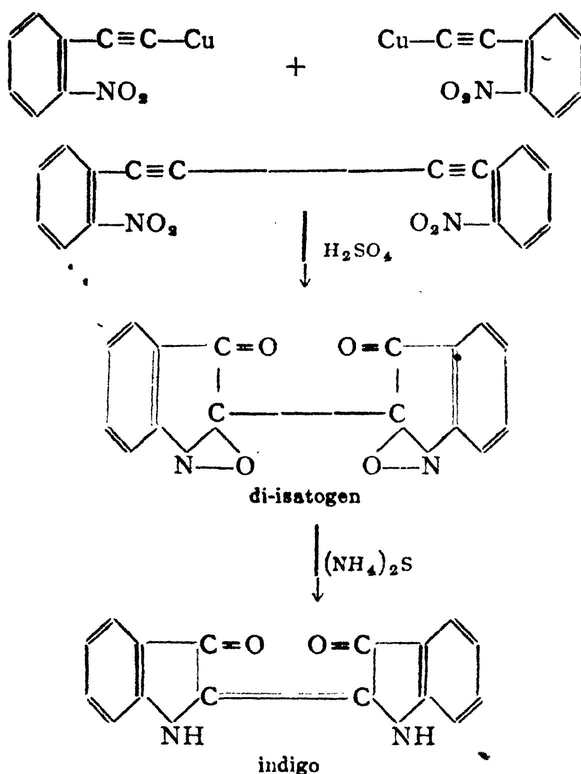
(Condensation of the two isatinyl residues and their partial reduction are involved in the above reaction. Originally, Baeyer used P· and PCl_3 and acetyl chloride to convert isatin into indigo).

That the isatin residues are united by carbon atoms is proved, by the conversion of di-(o-nitro-phenyl) diacetylene into indigo by the action of sulphuric acid and subsequent reduction. Baeyer obtained di-(o-nitro-phenyl) di-acetylene from o-nitro-cinnamic acid, as follows :—



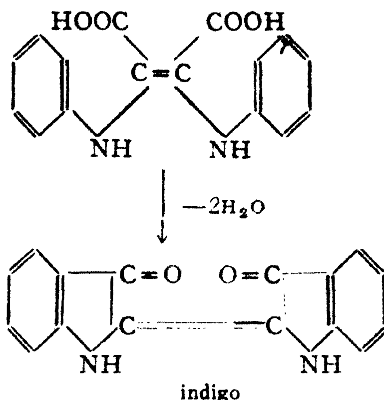
o-nitro-phenyl-acetylene
o-nitro-propiolic acid

The *o*-nitro phenyl-acetylene is converted into the corresponding copper salt by the action of ammoniacal Cu_2Cl_2. The copper salt is then oxidised with alkaline potassium ferricyanide (oxidative coupling) to give the di-acetylene derivative, which is then converted into indigo.



Indigo is also obtained by the alkaline reduction (glucose and alkali) of *o*-nitro propiolic acid; the latter is first hydrated across the triple bond to give *o*-nitro-benzoyl-acetic acid, which is subsequently reduced to indigo.

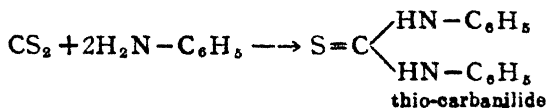
Also, the following synthesis by Salmony and Simonsen goes to prove the above structure for indigo. It consists in the elimination of a molecule of water from di-anilino-maleic acid, by fusion with $NaNH_2$.



indigo

The presence of *NH* group is indicated by the formation of *N*-methyl derivatives with methyl iodide or dimethyl-sulphate and alkali. On hydrolysis, the methyl indigo loses its nitrogen atoms as methylamine, CH_3NH_2.

SANDMEYER'S SYNTHESIS :—In this synthesis, the starting materials are aniline and carbon disulphide which react to form thio-carbanilide (diphenyl-thiourea) :

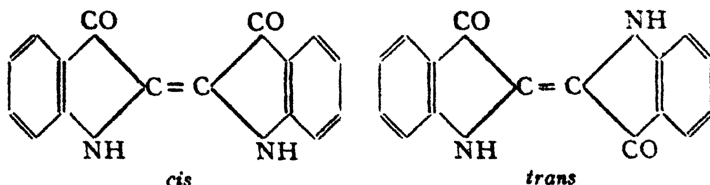


$$CS_2 + 2H_2N-C_6H_5 \longrightarrow S=C\begin{matrix} HN-C_6H_5 \\ HN-C_6H_5 \end{matrix}$$

thio-carbanilide

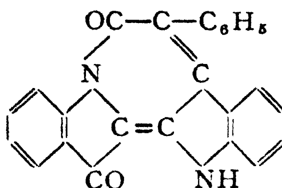
On heating with basic lead carbonate and potassium cyanide, sulphur is eliminated and hydrocyano-carbo-diphenyl imide is obtained. The latter is reduced with ammonium sulphide to thio-amide, which on heating with concentrated sulphuric acid, is converted into α-isatine-anilide. The anilide, on reduction with ammonium sulphide, gives indigo. This synthesis is of theoretical interest only.

Since the above formula for indigo contains a C=C linkage indigo might exist in *cis* and *trans* isomeric forms :—



cis *trans*

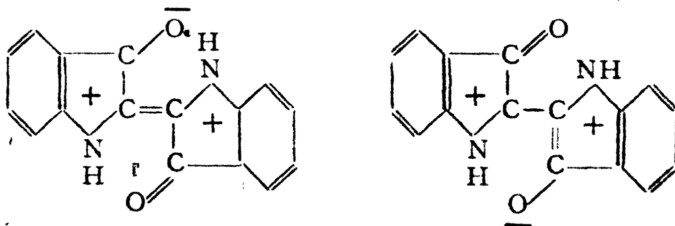
The *trans* form which would be the more stable of the two, is now accepted by some as the true structure for indigo. This is also in good agreement with the condensation of indigo with phenyl-acetic ester, $C_6H_5.CH_2.COOC_2H_5$ to form red a dyestuff of the formula :—



Even a di-derivative with phenyl-acetyl chloride, has been obtained. Such a ring closure is difficult to conceive with the *cis* form. X-ray studies also reveal the presence of a centre of symmetry in the indigo molecule. Hence it must be the *trans* form. Finally, a *trans* configuration is greatly stabilised by hydrogen bonding (O→HN).

However, recently, an oxalyl derivative of indigo a yellow dye has been obtained, by boiling indigo in nitrobenzene with oxalyl chloride. This indicates the earlier *cis* form ; probably in boiling nitro-benzene, there may be a change in the configuration of the form.

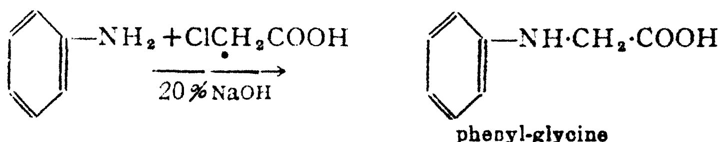
Colour of Indigo : Ene–dione system present in the indigo molecule is not alone responsible for the deep blue colour. Quinones containing this system are pale yellow. It is therefore proposed that indigo exists in several *ionic* resonance forms which contribute to the depth of the colour. Some of the ionic forms are :



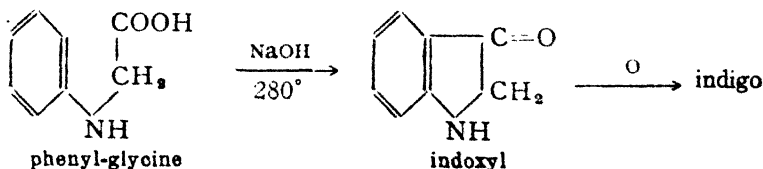
These molecules contain extended conjugated systems.

Commercial Synthesis of Indigo. The foregoing syntheses cannot be used for the production of indigo on a commercial scale. The natural indigo is obtained by the oxidation of indoxyl, present as the glucoside, *indican*. Indoxyl had been obtained by Baeyer from indigo and its structure established as β-hydroxy-indole. It is also isomeric with oxindole. The industrial chemists, therefore, turned their attention to a cheap synthetic production of indoxyl from coal-tar. Naturally, the coal-tar crudes should constitute the starting materials. Success has been achieved by Heumann who has thus developed two commercial syntheses. The synthetic product obtained by either of these methods has completely displaced the natural product from the market. The two syntheses are :—

HEUMANN'S FIRST SYNTHESIS :—The starting point is aniline. It is condensed with chlor-acetic acid to give phenyl-glycine.



Phenyl-glycine, on fusion with alkali, forms the alkali salt of indoxyl which is rapidly oxidised to indigo on æration of the alkaline melt.



As the reactions involve high temperature, partial decomposition of the raw material takes place and also the water formed in the reaction brings about partial hydrolysis of the phenyl-glycine. Hence, the yields of indigo are small.

Recently, the above method has been modified to give better and improved yields ; the phenyl-glycine is condensed with a mixture of sodium hydroxide and sodamide at 180–210°. Thus, the temperature range of the reaction is lowered and the water formed is also removed by sodamide :—

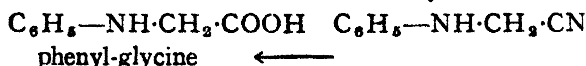


$$NaNH_2 + H_2O \longrightarrow NaOH + NH_3$$

Phenyl-glycine is now cheaply obtained from aniline by condensing it with formaldehyde and hydrocyanic acid, and subsequent hydrolysis of the nitrile :—



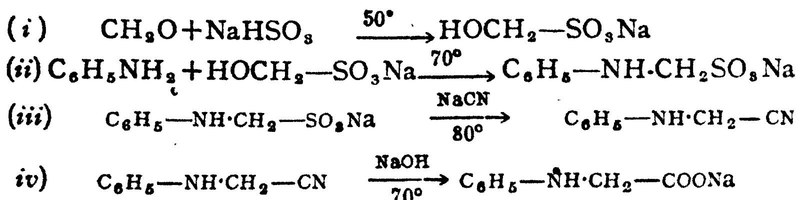
$$C_6H_5NH_2+OCH_2 \rightarrow C_6H_5N=CH_2$$



$$C_6H_5—NH·CH_2·COOH \leftarrow C_6H_5—NH·CH_2·CN$$

phenyl-glycine

Recently, a cheaper method, which is a modification of the above and which avoids the use of the poisonous HCN, has been developed. The essential steps involved are :



(*i*) $CH_2O+NaHSO_3 \xrightarrow{50^\circ} HOCH_2—SO_3Na$

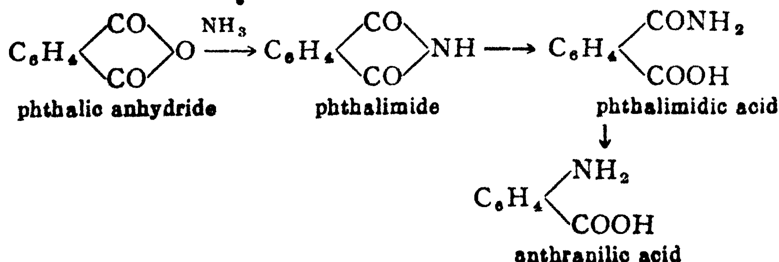
(*ii*) $C_6H_5NH_2+HOCH_2—SO_3Na \xrightarrow{70^\circ} C_6H_5—NH·CH_2SO_3Na$

(*iii*) $C_6H_5—NH·CH_2—SO_3Na \xrightarrow[80^\circ]{NaCN} C_6H_5—NH·CH_2—CN$

iv) $C_6H_5—NH·CH_2—CN \xrightarrow[70^\circ]{NaOH} C_6H_5—NH·CH_2—COONa$

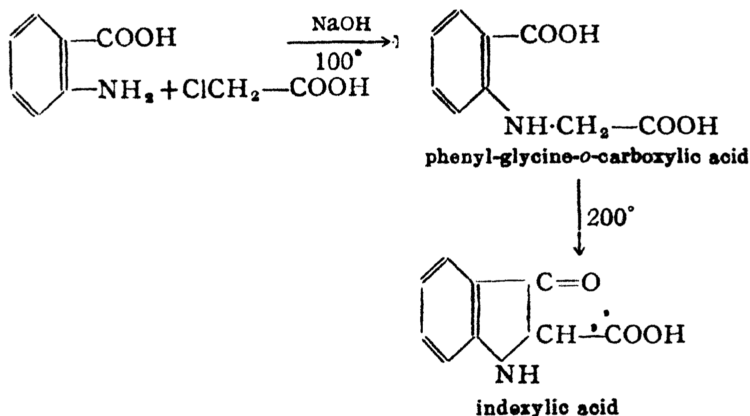
Aniline is also produced now from chlorobenzene by the action of ammonia under pressure. This method is claimed to give better yields and a pure product than the reduction of nitrobenzene.

HEUMANN'S SECOND SYNTHESIS :—This synthesis starts from anthranilic acid, which is obtained from naphthalene. Naphthalene is oxidised by the vapour-phase oxidation method, in presence of vanadium pentoxide (V_2O_5) to phthalic anhydride quantitatively. The older method of oxidation with fuming sulphuric acid in presence of mercury as a catalyst usually involved some charring and formation of sulphonated products, and hence it is discarded. The phthalic anhydride is converted into phthalimide by the action of ammonia or heating with urea at 130-140°. The imide is then changed into anthranilic acid by the action of alkaline hypochlorite (*Ca*-hypo-chlorite).



$C_6H_4(CO)_2O$ (phthalic anhydride) $\xrightarrow{NH_3}$ $C_6H_4(CO)_2NH$ (phthalimide) $\longrightarrow$ $C_6H_4(CONH_2)(COOH)$ (phthalimidic acid) $\downarrow$ $C_6H_4(NH_2)(COOH)$ (anthranilic acid)

Anthranilic acid is condensed with chloracetic acid to give phenyl–glycine–*o*–carboxylic acid. The latter, on fusion with alkali (*KOH*), forms indoxylic acid quantitatively.



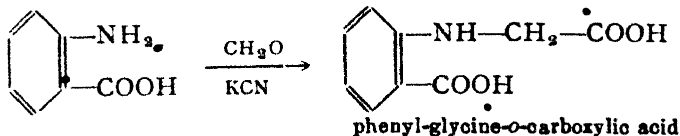
phenyl-glycine-*o*-carboxylic acid

indoxylic acid

(The presence of the *COOH* group in *ortho*–position to the amino group considerably facilitates the formation of the indole system).

The indoxylic acid which is a β ketonic acid then loses carbon dioxide and forms indoxyl which is rapidly oxidised to indigo, by ærial oxidation.

Phenyl glycine–*o*–carboxylic acid is now obtained from anthranilic acid by treatment with formaldehyde and potassium cyanide and subsequent hydrolysis of the nitrile formed.

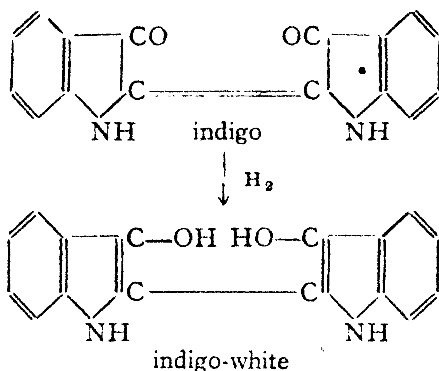


phenyl-glycine-*o*-carboxylic acid

Both these syntheses are now worked industrially to obtain synthetic indigo; the latter is a dark–blue powder mp. 390–392° insoluble in water, ether or alcohol, it dissolves in nitrobenzene and aniline. It can be sublimed under reduced pressure.

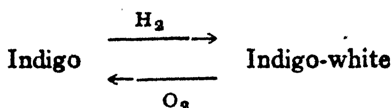
Dyeing with indigo : As indigo is insoluble in water, it is to be applied to fabrics by a special process. In the olden days, the dye was

fermented in presence of starchy matter to give a "vat"; the fabric to be dyed was then introduced into the vat and then exposed to air. The process was known as 'vat dyeing' and probably involved reduction by microbial action. At present, the vat is prepared by a chemical method, which is applicable to indigo and other allied dyestuffs. The principle of the process is that the vat dyes which are highly coloured and insoluble are first reduced to colourless (leuco) compounds soluble in aqueous alkali. Hence, the reduction is carried out in big vats by alkaline reducing agents. The most commonly used reducing agents are:—Sodium hydrosulphite, (*hydros*) $Na_2S_2O_4 \cdot 2H_2O$; calcium hydrosulphite, $CaS_2O_4 \cdot 2H_2O$ and formaldehyde-sulphoxylate or *rongalite*, $CH_2 \cdot OH \cdot OS \cdot ONa \cdot 2H_2O$. The chemical change involved is the formation of phenolic groups by reduction of the CO group to $C—OH$. Thus, in the case of indigo we have:



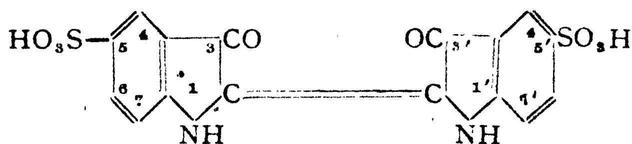
Indigo white is a crystalline compound soluble in hot water and alcohol. It dissolves in alkali with a yellow solution. The use of the above type of reducing agents limits the reduction of the dye to the formation of the leuco compound only. The fabric to be dyed is then immersed into the alkaline solution of the reduced dye in the vat. On exposure to air, the leuco compound is re-oxidised to the original dye in the interstices of the fabric. Hence, it is firmly held and is fast to washing and light.

Thus, this method of dyeing is limited to those dyes which are insoluble, but which are readily reduced to leuco compounds and which can be re-oxidised by air to the original dye. The changes entailed in the whole process are reversible.



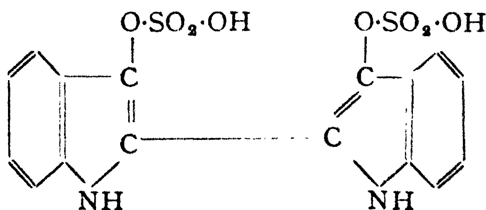
There is thus a colour change in vat dyeing.

This method of dyeing with indigo cannot be applied to the dyeing of woollen fabrics, as the latter are very sensitive to alkali. Woollen fabrics are therefore dyed with indigo-carmine. It is the sodium salt of 5-5′ disulphonic acid of indigo. It is obtained by sulphonating indigo with fuming H_2SO_4 and subsequent conversion into the Na-salt by one of the usual methods. It is a beautiful blue dye, very soluble in water and hence is used for wool dyeing. The structure of the disulphonic acid is:



N. B.: Positions 5-5′ are most reactive, hence they are first attacked, positions 7-7′ are next attacked in any direct substitution reaction. Positions 4 and 6 are not at all accessible for direct substitution.

A third and more useful method developed for the application of indigo is the "indigo sols" method or the solubilised vat dye method. In this method, the soluble sulphuric esters of indigo-white and its derivatives are finding application. They are obtained either by the action of chloro-sulphonic acid on indigo-white in presence of pyridine or by the action of chloro-sulphonic acid and pyridine, on indigo in presence of a suitable reducing agent. They possess the structure:



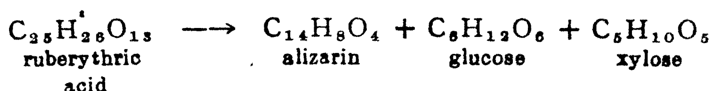
They are then converted into their Na-salts by the action of NaOH. The Na-salts are used for dyeing.

The fibre is introduced into the sol and the dye is then developed on it, by acid hydrolysis of the ester to the leuco compound, followed by oxidation to indigo; the oxidation is effected by sodium nitrite. The practical advantages of the sols are: *(i)* they are soluble in water, *(ii)* they are readily absorbed by the both the cotton and woollen fabrics, *(iii)* the method is applicable to all other types of vat dyes.

The Tyrian purple or Royal purple, the imperial dye of the Romans, is proved to be the 6-6′ dibromo-indigo. Friedlander isolated 1·4 g. of the dye from 12,000 molluscs. Sachs has reported its synthesis from *p*-bromo-*o*-nitro-benzaldehyde; the latter is condensed with acetone in presence of glucose and alkali. At present this dye is not manufactured, but the 5-5′, 7-7′, tetra bromo derivative, obtained by the direct bromination of indigo and known as the Ciba blue 2B, constitutes an important vat dye.

Alizarin

Alizarin is the red dye from the madder root; it is present as the primeveroside, ruberythric acid (see p.). On acid or enzymatic hydrolysis, ruberythric acid gives alizarin, glucose and xylose.



Alizarin is one of the most important mordant dyestuffs belonging to the anthraquinone type of dyestuffs. Its constitution is based on the following evidence:

(*a*) Its molar composition is $C_{14}H_8O_4$.

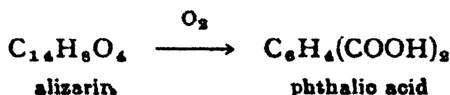
(*b*) On distillation with zinc dust, alizarin gives anthracene.

This indicates the presence of the anthracene nucleus in alizarin; thus alizarin is an oxygenated derivative of anthracene. Anthraquinone is also an oxygenated derivative of anthracene. A comparison of the molecular formulas of these compounds,

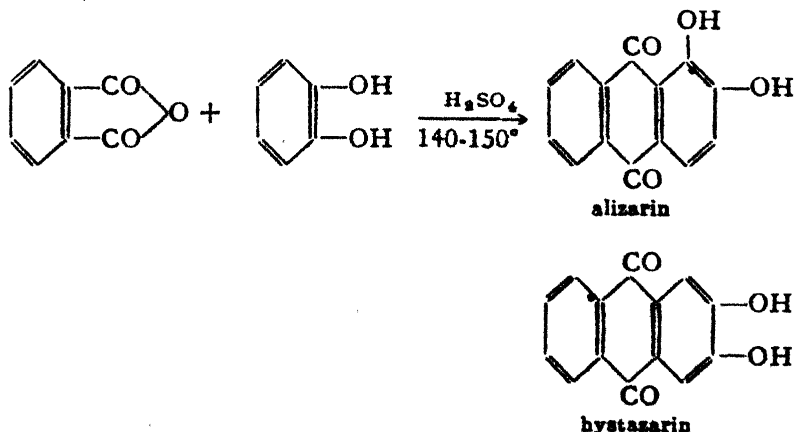


clearly suggests that the same carbon framework is present in all the three compounds. Further, alizarin contains *two* atoms of oxygen more than anthraquinone. Now, alizarin is soluble in alkali, (anthraquinone is not soluble), which indicates the presence of phenolic *OH* groups. On acetylation with $(CH_3CO)_2O$, alizarin gives a di-acetyl derivative. Hence, alizarin may be a dihydroxy derivative of anthraquinone. Graebe and Liebermann converted anthraquinone into a dibromo-derivative which, on fusion with alkali, gave alizarin. They, thus, proved *synthetically* that alizarin was dihydroxy-anthraquinone. But the positions of the *OH* groups are not fixed by this synthesis. This is settled by the following considerations :—

On vigorous oxidation, alizarin is converted into phthalic acid :—



This result indicates that both the *OH* groups are on the same nucleus which disappears on oxidation. Their relative positions are shown by the synthesis of alizarin from phthalic anhydride and catechol; some isomeric hystazarin is also formed.



Both the hydroxyl groups are thus present in the same nucleus (derived from the catechol molecule) and are in ortho positions to each other.

The above evidence limits the structure of alizarin to two possible formulas I and II.

I II

The choice between the two is made as follows: Two mononitro-alizarins are known, both of which on oxidation give phthalic acid only. This indicates that the NO_2 group and the OH groups are present on the same nucleus. Now it is only the formula I that admits of two such mono-nitro derivatives. Hence alizarin is assigned the formula I.

Relation of alizarin to purpurin can also be used to decide between the two formulas. Alizarin, on oxidation with MnO_2 is converted into purpurin. The constitution of purpurin follows from its synthesis from quinizarin.

$\xrightarrow{O_2}$

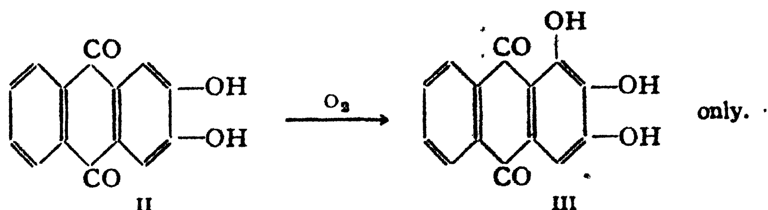
quinizarin purpurin

Only formula I, can give rise to purpurin and an isomeric compound III. Formula II can form only the compound III.

$\xrightarrow{O_2}$ +

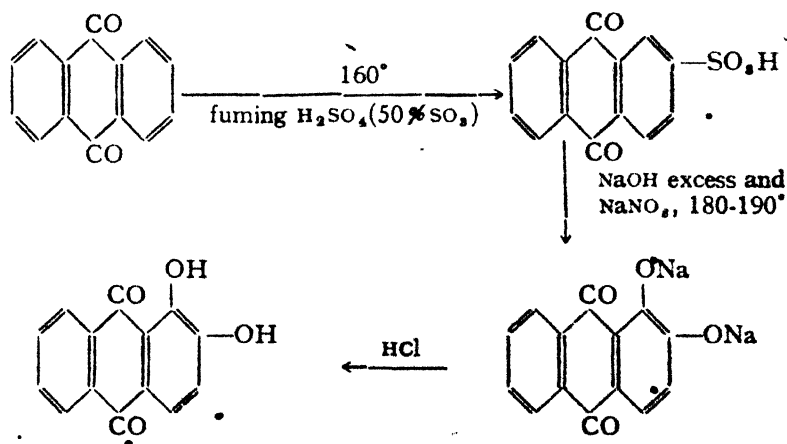
purpurin anthragallol

I III

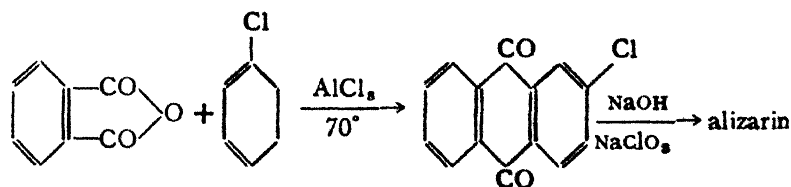


only.

COMMERCIAL SYNTHESIS OF ALIZARIN :—This synthesis was achieved simultaneously by Perkin, and Graebe and Liebermann. The starting-point is anthraquinone. It is sulphonated to give the anthraquinone-β-sulphonic acid. The latter, on fusion with alkali in presence of an oxidising agent *e. g.*, $KClO_3$ or $NaNO_3$ gives sodium alizarate. The function of the oxidising agent is to remove the H_2 formed during the reaction and thus increase the yield of the product. On acidification, alizarin is formed. It is further purified through the sparingly soluble Ca-salt.



Recently, a process has been developed starting from phthalic anhydride; the essential steps are :



(The *o*-benzoyl-benzoic acid derivative formed as the first step in the process is cyclised with H_2SO_4 at 150°).

Alizarin forms dark-red prisms, m. p. 290°. It is sparingly soluble in water, but soluble in alcohol and in alkali. It is used as a mordant dye. It forms metallic alizarates which are coloured and insoluble; they are called "lakes." The colour of the lake depends on the nature of the metal. Thus alizarin gives :

with Al $(OH)_3$ ⟶ red lake (Rose red)

with Cr $(OH)_3$ ⟶ maroon lake (brown-violet)

with Fe $(OH)_3$ ⟶ violet lake (Brown-black)

with Ca $(OH)_2$ ⟶ blue lake (purple red)

Hence, alizarin is called a poly-genetic dye i.e gives different shades with different mordants. The stability of the lakes is due to the presence of a chelate ring

CHAPTER VII

VITAMINS AND HORMONES

Vitamins and hormones are natural organic compounds which possess a marked physiological activity and a high specificity of function. They are both required in small quantities and are essential for the maintenance, growth and well-being of all animal organisms. But there is no structural similarity between the two classes of compounds. On the other hand, the distinction between the two classes is not very sharp. However, the only difference is that the vitamins are usually supplied to the animal organism through the intake of foods, while the hormones are elaborated in the body of the animal organism by the ductless glands, *e. g.*, the thyroid and pituitary glands. Recently, it has been shown that some members belonging to the vitamin B complex, are synthesised in certain animals through the agency of bacteria.

VITAMINS

Introduction :—About 1890, Eijkmann made the accidental discovery that *beri-beri* could be produced experimentally in fowls, fed on polished rice only, and he directed his earliest work on the isolation of the anti-neuritic substance. It was then becoming known that the diseases like *beri-beri*, *scurvy* and *rickets* were caused by the deficiency of some accessory food-factors and could be prevented or cured by dietary measures. At the same time, Hopkins, as a result of large amount of experimental work, showed that small quantities of substances other than proteins, carbohydrates, fats and mineral salts were absolutely essential for life. The normal development of the animal organism, thus, requires food which consists of fats, proteins, carbohydrates, mineral salts and small quantities of food accessories. The lack of the '*accessories*' in the diet causes '*deficiency diseases*' (avitaminosis). Funk (1912), because of their vital importance to life and his belief that they were amino compounds, called these food accessories (Vital amine)—*Vitamines*. Later researches, however, revealed that they were not necessarily aminoderivatives and hence, the name was modified to *Vitamins*. The vitamins possess a relatively complex structure and many of them are unstable towards heat. The animal organism is incapable

of synthesising them from simple substances. They are, therefore, to be supplied through food. A rational and well-balanced diet must contain a sufficient quantity of all the vitamins.

The vitamins are widely distributed in nature, in the plant materials; they are found in the animal organism only as a result of food intake.

The following is a list of some important and common vitamins with their sources.

Vitamin	Source
Vitamin A Vitamin A_2	Fish-liver oils
Vitamin B_1 Vitamin B_2 Vitamin B_6 Nicotinic acid Pantothenic acid	Rice polishings, yeast, whey, egg etc.
Vitamin C	Citrus fruits, green vegetables, chillies, cabbages etc.
Vitamin D_1 Vitamin D_2 Vitamin D_3	Cod-liver oils, ergosterol.
Vitamin E α-Tocopherol β-Tocopherol	Wheat germ oil.
Vitamin H (Biotin)—	Kidney, liver and egg.
Vitamin K_1 and K_2	Cereals, leafy tissues, hog-liver.
Vitamin P (citrin)	Grape fruit, orange, lemon etc.

There are some compounds known which are related to the vitamins; they have no biological activity, but are transformed *in vivo* into the active vitamins. They are called 'pro-vitamins'. β-carotene is pro-vitamin *A* and ergosterol is the pro-vitamin D_2.

Classification and Nomenclature:—So far, 22 to 25 vitamins have been recognised; they are divided into two classes: (*a*) the fat-soluble vitamins and (*b*) the water-soluble vitamins.

They are commonly designated by letters of the alphabets *e.g.* vitamin A, Vitamin B, etc. The components of an individual vitamin, if any, are further indicated by subscripts, *e.g.*. Vitamin B_1, Vitamin B_2, etc.; the discovery of the existence of these substances, their concentration, isolation and purification depend on the development and growth of biochemical methods and tests. It is a close co-operation between the organic chemist and the biologist, that has produced such excellent and far-reaching results. The syntheses of these highly complex compounds in the laboratory represent the highest form of chemical ingenuity.

General Characteristics:—The vitamins possess a number of common properties, which justifies their being grouped together. These properties are: (*i*) the vitamins are required only in very small quantities: (*ii*) they cause marked physiological effects; (*iii*) they are characterised by specificity of function. Thus each vitamin is related to a definite disease; the lack of the vitamin causes the disease and the cure is effected by the supply of the same vitamin; (*iv*) they are generally complex in nature; a few exceptions are: nicotinic acid and *p*-amino benzoic acid; (*v*) animals appear to depend on plants for the supply of vitamins. (*vi*) they do not serve as sources of energy. (*vii*) they cannot be synthesised by the animal organism. (*viii*) their action is catalytic.

Detection and estimation of vitamins:—A number of reactions have been developed and standardised for the detection and estimation of the common vitamins.

Vitamin *A* ... ... Intense blue colouration with $SbCl_3$ in $CHCl_3$ solution.

Vitamin B_1 ... ... Estimated by lumitron (photoelectric fluorimeter).

Vitamin *C* ... ... Powerful reducing action; reduction of Ag salt and of 2, 6-dichlorophenol-indophenol.

Vitamin *D* ... ... Possesses a characteristic ultra-violet absorption spectrum.

These reactions in conjunction with the results of animal control experiments in the case of vitamins B_1, B_2, E, K_1, K_2 etc. have helped in the isolation of the vitamins in the pure state from different sources. We shall now discuss the constitution and syntheses of a few important and common vitamins.

VITAMIN A

Occurrence :—Vitamin *A* is a fat-soluble, growth promoting vitamin and is present in fats like butter, in blood and also in the livers of fish *e. g.* cod and halibut. The cod-liver and halibut liver oils are rich sources of this vitamin. The liver oils of the sharks from the Indian seas, have also been found to be a very rich source of this vitamin. The liver oil of the saw-fish also contains this vitamin. This vitamin is also present in egg yolk. Other sources are sweet potatoes, tomatoes, liver, asparagus etc.

A second complex vitamin A_2 has been detected in certain fish oils, especially in those from some fresh-water fishes. Structurally it is similar to vitamin *A*, but contains six conjugated systems.

Isolation :—The vitamin is isolated from the unsaponifiable parts of animal fats, especially liver oils. The fat is hydrolysed with alcoholic KOH in an inert atmosphere, in presence of a small amount of hydroquinone when the vitamin remains in the unhydrolysed residue. The isolation of the vitamin is then effected by one of the three different methods: (*i*) fractional crystallisation at low temperatures, (*ii*) chromatographic adsorption and (*iii*) molecular distillation.

In the first method, the unsaponifiable matter is dissolved in methanol, and the solution is chilled to—70°, when the sterols *e. g.* cholesterol etc. crystallise out. Further purification is brought about by fractional distillation in vacuo.

In the chromatographic adsorption method, the unsaponified matter is adsorbed on aluminium oxide, which is followed by differential adsorption on calcium hydroxide. This method gives a preparation of the highest purity. This method however has the disadvantage that during the adsorption procedure, some destruction of the vitamin takes place.

The last method namely, the molecular distillation method has rendered possible the isolation of the vitamin and its esters in a relatively purer form; the unsaponified matter is dissolved in a mixture of neutral residue oil and constant yield oil, and distilled stepwise, over long ranges of temperatures.

The vitamin *A* is finally crystallised from ethyl–formate or from propylene oxide. Pure vitamin *A* crystallised from ethyl–formate forms yellow needles which melt at 63 to 64°.

DETECTION AND ESTIMATION—Carr and Price have developed a method for the qualitative and quantitative determination of vitamin *A*. It is based on the reaction of this vitamin with antimony chloride. When the vitamin is brought into contact with antimony chloride in $CHCl_3$ solution, a deep–blue colouration is formed. The measurements are made with the Lovibond tintometer. The results are expressed either in the B. V. (blue value units) or in the C. L. O. (cod–liver oil units). The above reaction, however, is not peculiar to vitamin A, but is given by all carotenoids. Recently, Rosenthal has reported that addition of guaiacol in the above Carr–Price reaction changes the blue colour to a relatively permanent reddish–purple which is specific for vitamin *A*. Recently, the fact that this vitamin gives an absorption band in the ultra–violet at 328 m. u., is used in its identification.

Composition and Structure:—The molecular composition of vitamin *A* is $C_{20}H_{30}O$. Its constitution is based on the addition and degradation reactions characteristic of such highly unsaturtated compounds. The typical and important reactions are addition reaction with hydrogen in the presence of catalyst and the degradation reactions with ozone, chromic acid or potassium permanganate. The following are some of the reactions of vitamin *A*:

(*a*) With *p*–nitro–benzoyl–chloride, a crystalline ester is formed; this indicates the presence of an alcoholic group. The presence of a primary alcoholic group is further indicated by the formation of an aldehyde on oxidation with MnO_2 or H_2O_2.

(*b*) AMOUNT AND LOCATION OF UNSATURATION.

Vitamin A, on catalytic hydrogenation in presence of Pt, or on reduction with Al–amalgam gives perhydro vitamin A

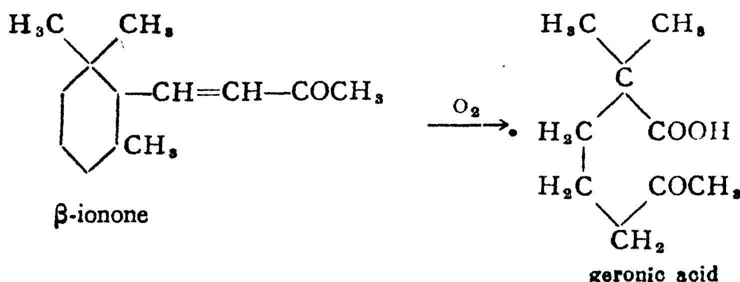


$$C_{20}H_{30}O \xrightarrow{H_2} C_{20}H_{40}O$$

∴ The vitamin A molecule contains five double bonds and one ring.

(c) On oxidation with ozone, geronic acid is formed; one molecule of the latter being obtained from one molecule of the vitamin.

Hence, the vitamin must contain one β–ionone residue. For we have :—



(*cf.* the reaction of ozone with β-carotene; it gives two molecules of geronic acid).

The β ionone unit contains 10 carbon atoms and the remaining ten carbon atoms are probably present as a polyene system. The latter may be built up of two isoprene units. This suggests the presence of two lateral methyl groups. Vitamin A on alkaline oxidation with $KMnO_4$ gives two moles of acetic acid per one mole of the vitamin. Hence the presence of two isoprene units is confirmed.

N.B.—The Kuhn-Roth method for the determination of C-methyl groups (see p. 449) indicates the presence of three C-methyl groups: two from the polyene side-chain and one from the β-ionone nucleus.

Lastly Heilbron obtained, by the action of HCl on the vitamin, a product which is anhydro vitamin *A*. The latter, on dehydrogenation with Se, gave 2·6 dimethyl-naphthalene. (*cf.* the behaviour

of carotenoids : p. 448). These results thus confirm the presence of two isoprene units.

These isoprene units must be present as a side-chain on the ionone nucleus. Hence, the probable formula for vitamin *A* is:—

```
H3C   CH3
   \ /
    C                  CH3                  CH3
   / \                 |                    |
H2C   C—CH=CH—C=CH—CH=CH—C=CH—CH2OH
 |    ||     \_____________/   \______________/
H2C   C—CH3   isoprene unit     isoprene unit
   \ /
   CH2
```

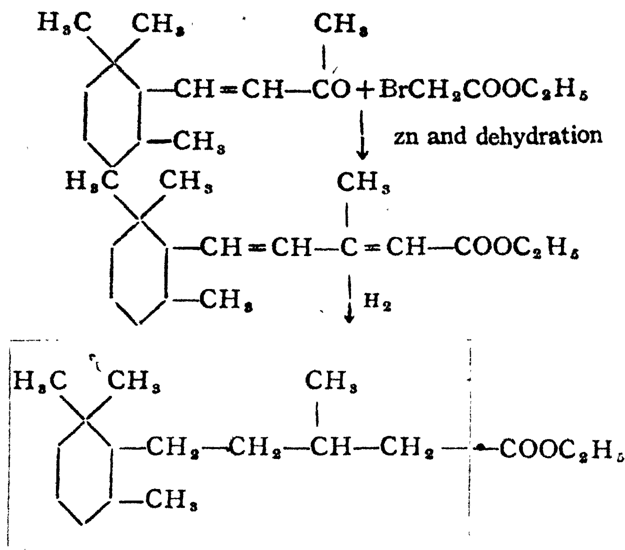
This formula was proposed by Karrer, Morf and Schopf.

RELATION TO β-CAROTENE :—Further, the above formula indicates a close relationship to β-carotene. The molecule of the latter can be imagined to undergo splitting at the central double bond to form a vitamin A molecule.

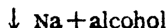
The above relationship between vitamin *A* and β-carotene is experimentally established. When β-carotene is given in conjunction with vitamin D to an animal suffering from vitamin *A* deficiency, the effects are the same as when cod-liver oil, *i.e.* vitamin *A* is administered. In the living organism, β-carotene is converted into true vitamin *A* which is then stored up in the liver. Vitamin *A* and β-carotene are inter-changeable in the animal diet. Hence, β-carotene is called "pro-vitamin *A*" *i.e.* it is the precursor of the vitamin *A* in nature. Recently, this conversion of β-carotene into vitamin *A* by chemical methods has been reported. β-Carotene is oxidised with H_2O_2 in glacial acetic acid to form the vitamin *A* aldehyde; the latter on reduction with Al-iso-propoxide gives vitamin *A* (alcohol).

Synthesis of Perhydro-vitamin A :—The above structure for the vitamin, was established conclusively by Karrer by synthesising perhydro-vitamin *A* ; it is the reduction product of vitamin *A*. This synthesis establishes definitely the nature of the C-skeleton of the

molecule. The starting-point in the synthesis is the β-ionone unit. The different steps involved are :

















which on hydrolysis and heating gives :—



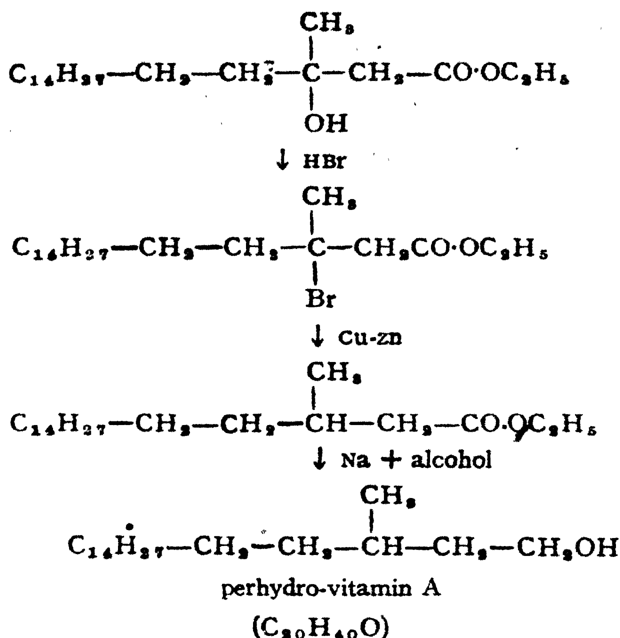




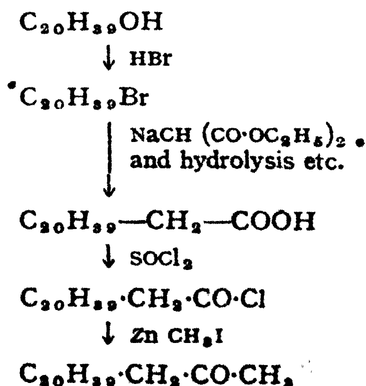








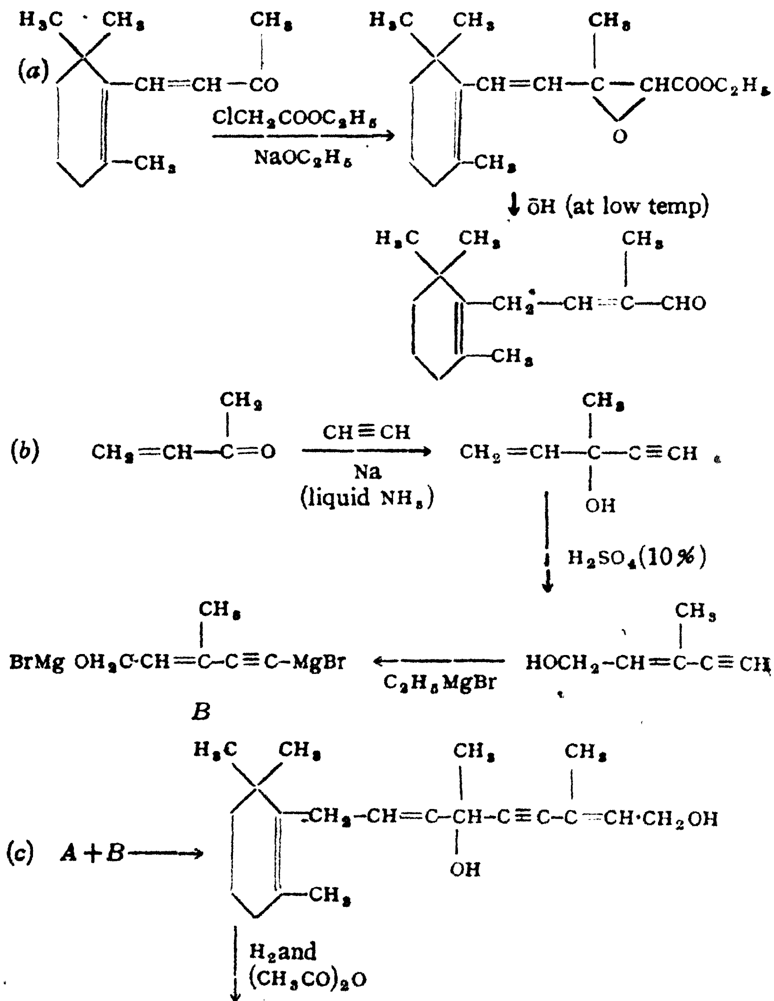
It is a liquid and is identical with the liquid obtained by the hydrogenation of natural vitamin A concentrate. Karrer, further, confirmed the identity by subjecting the *synthetic* perhydro-vitamin A and the one obtained from the natural product to the following series of reactions :—

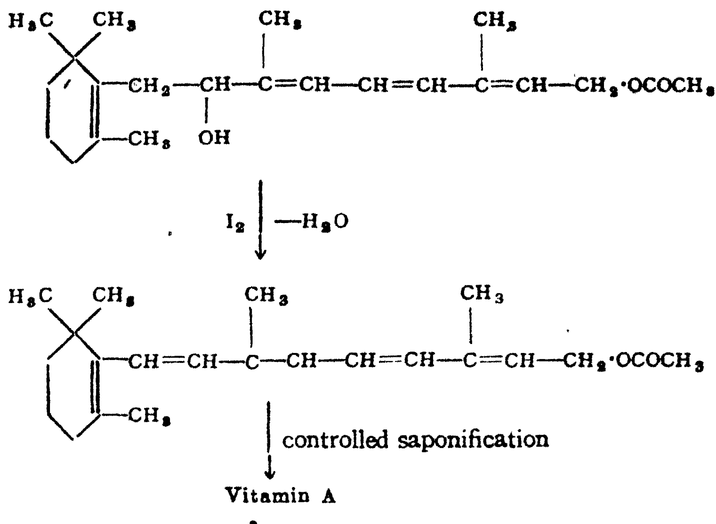


The melting-points of the acid and of the semi-carbazone of the ketone obtained as above, were identical in both the series.

Recently, syntheses of pure vitamin A, on a commercial scale, have been announced. They are due to *(i)* Isler, *(ii)* Warner and *(iii)* Warner and Milas. In all these syntheses, the starting-point is β-ionone.

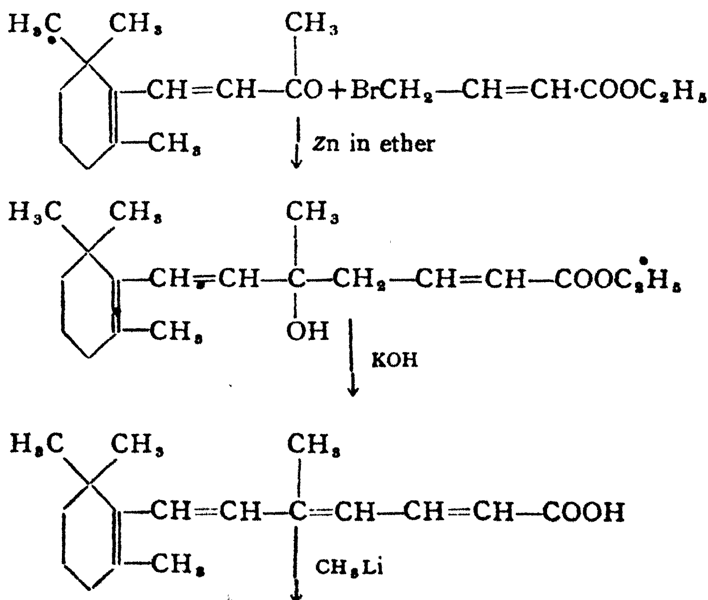
(i) *Isler's synthesis :*

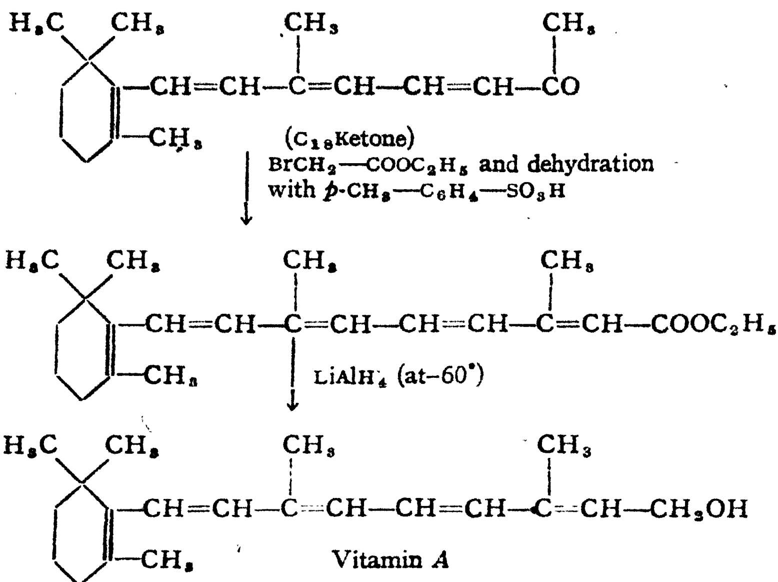




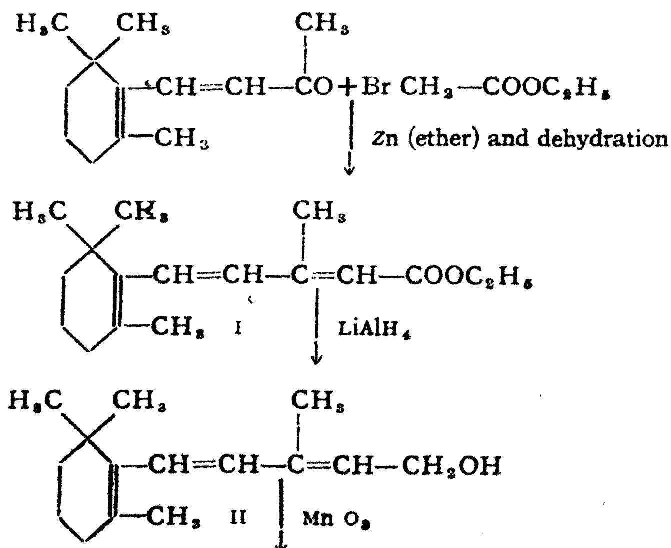
The vitamin A is isolated through the crystalline naphthoate.

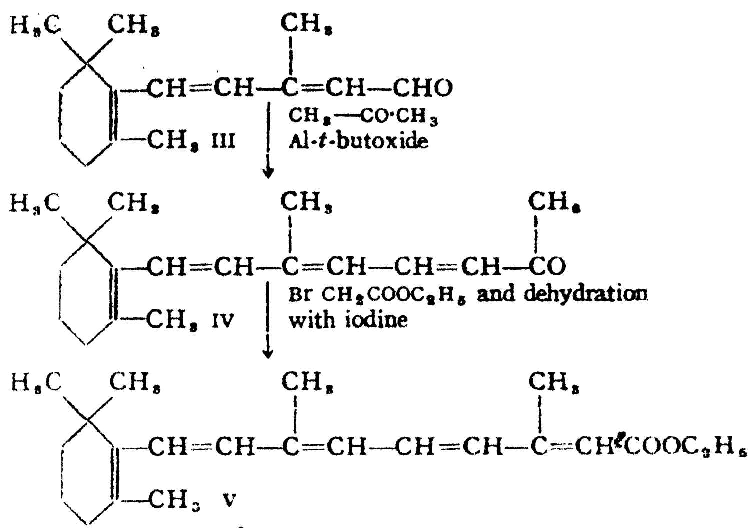
(ii) *Warner's synthesis :*





(iii) *Warner and Milas synthesis :*





The above ester on hydrolysis and subsequent reduction with $LiAlH_4$ at—60°, gives the alcohol, vitamin *A*.

In a recent modification of the above method, II is converted into IV in one step by the use of acetone and Al-t-butoxide. Both oxidation of CH_2OH group and subsequent condensation are effected in one step.

Vitamin B Group

This is a highly complex group of compounds possessing vitamin activity. So far the following members of this group have been isolated and studied in detail. They are all water soluble vitamins and are present in varying proportions in milk, yeast, vegetables, liver, etc. They are separated into (*a*) heat-labile and (*b*) heat-stable factors.

Vitamin B_1 or aneurin, or thiamin, ⟶ Heat-labile

Vitamin B_2 or riboflavin or lactoflavin,
Nicotinic acid, Nicotinamide,
Pantothenic acid,
Vitamin B_6 or pyridoxin, } ⟶ Heat-stable

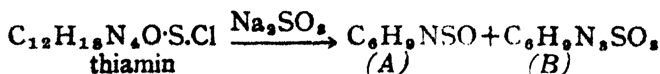
Vitamin Bc or folic acid,
Vitamin B_{12},
p-Amino-benzoic acid,
Inositol.

8

Vitamin B_1 :—Historically this is the first vitamin to be tackled and investigated. It is sometimes called the "morale" vitamin ; it is the less heat-stable component of "vitamin *B* complex." It occurs widely distributed in yeast, eggs, cereals and rice polishings ; (the last constitutes a rich source of this vitamin). It is rapidly destroyed by heat in the presence of alkalies. It is a water-soluble vitamin. According to Funk, it controls the carbohydrate metabolism, probably disposing of the lactic acid formed by muscular activity. It is the anti-neuritic vitamin and hence, called "*aneurin.*" It is also called thiamin. Like vitamin *A*, it is essential to life of higher animals. Its deficiency results in beri-beri in human beings and poly-neuritis in experimental animals. Beri-beri is prevalent in the East, where a diet of polished rice is very common.

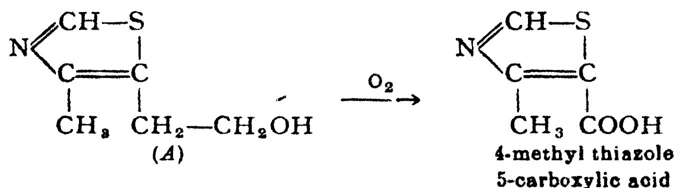
Isolation of the Vitamin :—The rice polishings constitute the source material; other sources are; fish, milk, tomatoes etc. the source material is extracted with acidulated water (pH 4·5), after addition of a small quantity of toluene to prevent fermentation. The vitamin being soluble in water, is present in the extract. The latter is filtered through a column of Fuller's earth which rapidly and quantitatively adsorbs the vitamin. The vitamin is then recovered by elution with a solution of quinine sulphate. Quinine displaces the vitamin which goes into solution ; excess of quinine is precipitated by the addition of Ba $(OH)_2$; the vitamin is then precipitated with $AgNO_3$ at pH 7·5 ; and regenerated from the silver salt by the addition of HCl. The vitamin in the form of its hydrochloride is recrystallised from alcohol. Recently, the mono-nitrate is used for the purification of the vitamin. Vitamin B_1 melts at 248-50°C with decomposition ; it is the chloride of a strong base and exists in the form of colourless, odourless crystals with a slight salty taste. It is hygroscopic and is soluble in water : the solution is acidic to litmus ; it is optically active.

Constitution of Vitamin B_1 :—Our knowledge of the structure of this vitamin is due to the researches of R. Williams and his collaborators. The hydrochloride of the vitamin is decomposed by Na_2SO_3 in aqueous SO_2 solution into two products. One containing the *thiazole* ring and the other *pyrimidine ring* :

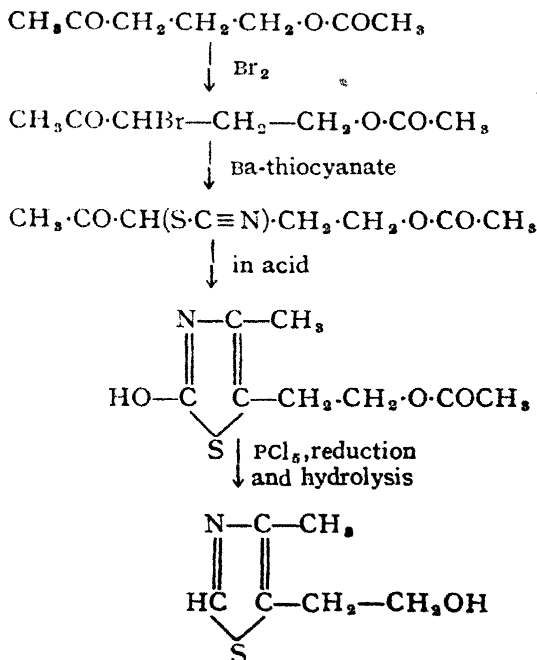


$$\underset{\text{thiamin}}{C_{12}H_{18}N_4O{\cdot}S.Cl} \xrightarrow{Na_2SO_3} \underset{(A)}{C_6H_9NSO} + \underset{(B)}{C_6H_9N_3SO_3}$$

NATURE OF (A) :—Oxidation of (*A*) with nitric acid, gives an acid with the composition $C_5H_5O_2NS$. This acid is identical with the compound previously obtained by Windaus and co-workers, by oxidation of the vitamin B_1 itself. It has been identified as 4-methylthiazole-5-carboxylic acid. Clarke and Gurin have proved that (*A*) is the corresponding 5-β-hydroxyethyl derivative :—



The presence of the thiazole nucleus in the molecule of the vitamin was at first accepted on the basis of the peculiar stability of the sulphur linkage and on the study of the ultra-violet absorption spectrum. Later on, the above structure for *A*, was confirmed by a synthesis by Andersag and Westphal.

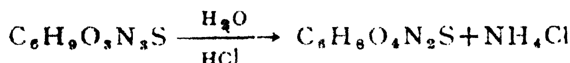


NATURE OF (B) :—The structure of this part is based on the following evidence :

(i) On heating it with water under pressure, H_2SO_4 is formed.

(ii) On fusion with alkali, alkali sulphite is obtained; these reactions indicate that it is a sulphonic acid.

(iii) On treatment with aqueous HCl, it gives rise to a compound, whose reactions suggest that it is a sulphonic acid and that it contains a hydroxyl group.



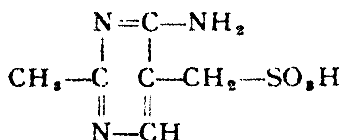
$$C_6H_9O_3N_3S \xrightarrow[HCl]{H_2O} C_6H_8O_4N_2S + NH_4Cl$$

The above conversion further indicates that an amino group is replaced by a hydroxyl group. Thus the presence of an amino group in *B* is established.

(iv) On reduction with *Na* in liquid NH_3, a base 2, 5 dimethyl 6 amino- pyrimidine is formed ; this shows the presence of a pyrimidine ring.

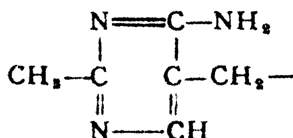
(v) Lastly Andersag and Westphal have shown that oxidation of vitamin B_1 with acid Ba $(MnO_4)_2$, gives 2—methyl-4-amino-5-amino methyl-pyrimidine.

Hence (*B*) must be represented by—

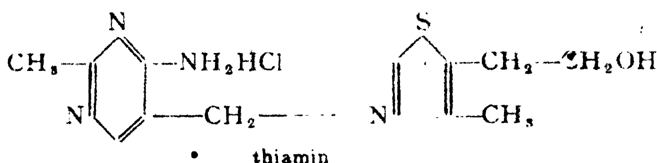


(sulphonic acid of 2-5 dimethyl-6-amino-pyrimidine).

This sulphonic acid is formed by the action of Na_2SO_3 on the vitamin ; the fundamental unit present must therefore be :—



Thus the vitamin B_1 molecule is built up of a thiazole nucleus and a pyrimidine nucleus. The mode of linking of the two units is established as follows : the original vitamin molecule does not contain a SO_3H group, but it appears in one of the products (*B*) of cleavage. The position of the sulphonic group therefore indicates the point of attachment of *B i. e.* the pyrimidine unit. The point at which the —CH_2—bridge is attached to the N-atom of thiazole nucleus, is suggested by the resemblance in behaviour of the vitamin, to quaternary ammonium salts *e. g.* the methiodide of 4 methyl-5-hydroxyl-ethy-thiazole, on potentiometric analysis. Hence vitamin B_1 has been assigned the structure :



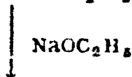
thiamin

Synthesis :—Thiamin is now obtained on a large scale by synthetic methods only ; in fact, its isolation from natural sources cannot compete with the synthetic processes. The synthesis consists of a (*a*) synthesis of the pyrimidine and the thiazole units and (*b*) the condensation of the two to give the vitamin.

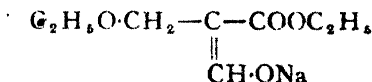
(*a*) Synthesis of the pyrimidine unit :

(i) 

$$C_2H_5O{\cdot}CH_2{-}CH_2{-}COOC_2H_5 + H{\cdot}COOC_2H_5$$

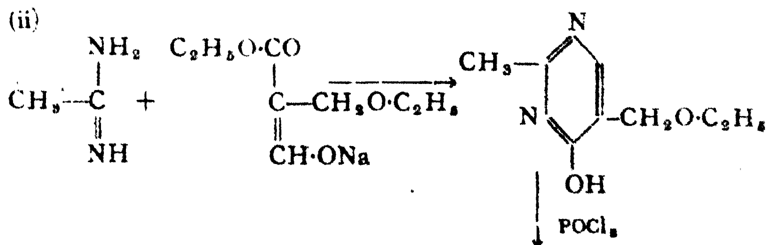


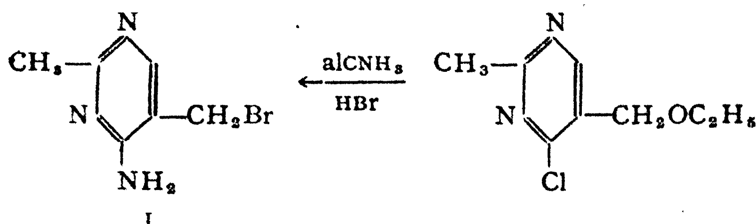
$\downarrow NaOC_2H_5$



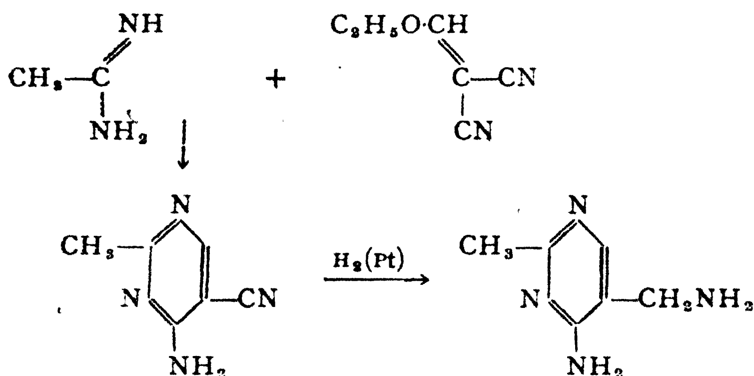
$$C_2H_5O{\cdot}CH_2{-}\underset{\underset{CH{\cdot}ONa}{\|}}{C}{-}COOC_2H_5$$

(ii)



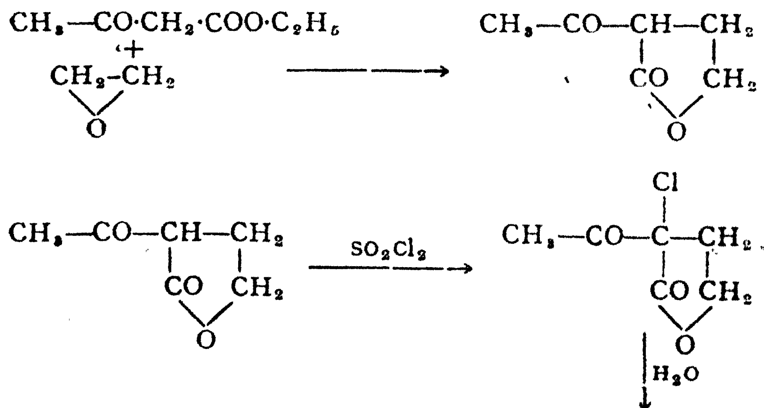


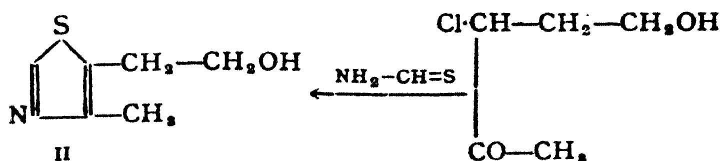
I is also obtained as follows :



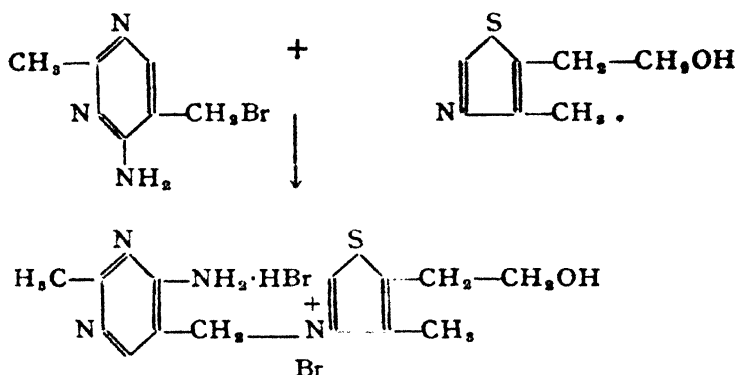
The latter on treatment with HNO_2 and subsequently with HBr gives I.

(b) Synthesis of thiazole unit (II).



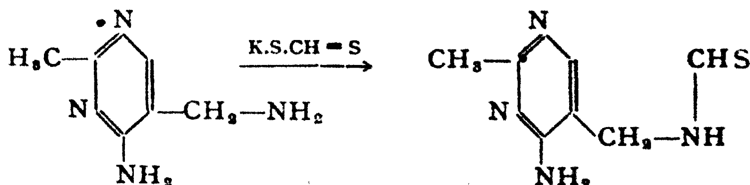


(c) Synthesis of the vitamin : I and II are condensed to give the bromide-hydrobromide;



The bromide–hydrobromide on boiling with AgCl in alcohol gives the chloride—identical with thiamin-hydrochloride.

Another synthesis with a slight modification of the above is also known ; a thio form amide containing a pyrimidine ring is used.



The latter is condensed with CH_3—CO·CHCl·CH_2—CH_2OH in alkaline condition to give the thiamin–hydrochloride. This synthesis is due to Todd and Bergal.

Vitamin B_2

Occurrence :—This vitamin occurs widely in plants and in animal organs. It is present in Warburg's yellow respiration (oxidation) ferment. It is yellow in colour, gives a yellowish-green fluorescence and is sensitive to light. Originally, it was known by different names, indicating the source; thus it was called ovo–flavin (from eggs), lacto–flavin (from milk) and hepaflavin (from liver). It is now called riboflavin as it contains the ribityl residue in its molecule.

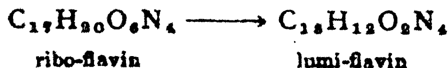
Isolation :—The richest natural source of this vitamin is the egg albumin; another important source is whey. The latter is acidified with concentrated HCl and the vitamin adsorbed on Fuller's earth. The adsorbate is washed free of acid and the vitamin eluted with a mixture of pyridine, methanol and water. The methanol is then removed from the eluate and the vitamin finally obtained by an elaborate procedure involving several precipitations and re–solutions; at one of the stages, the vitamin is thrown down as its sparingly soluble thallium salt. Lacto–flavin crystallises from alcohol in clusters of yellow-orange needles m. p. 182° (decom.). It is relatively stable to heat but deteriorates on exposure to light; it undergoes change in composition. In neutral medium, the ribose residue is completely eliminated, while in alkaline condition, *lumi-flavin* is formed, the ribose residue being replaced by—CH_3.

Constitution :—This was established by the brilliant researches of Kuhn, Karrer and their collaborators. It is based on the following analytical and synthetic evidence.

(*a*) The molecular composition is $C_{17}H_{20}O_6N_4$.

(*b*) On acetylation, it gives a tetra-acetyl derivative; this indicates the presence of four hydroxyl groups, which is in good agreement with its great solubility in water.

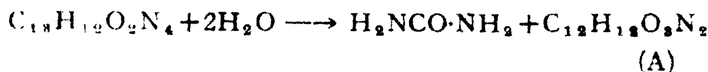
(*c*) On exposure to sunlight in alkaline medium it is changed into a yellow compound, lumi-flavin :



$$\underset{\text{ribo-flavin}}{C_{17}H_{20}O_6N_4} \longrightarrow \underset{\text{lumi-flavin}}{C_{13}H_{12}O_2N_4}$$

The decomposition thus involves the splitting off of a side-chain with the composition $C_4H_8O_4$. Further, lumi-flavin is insoluble in water and cannot be acetylated at all. These results therefore indicate that the side-chain which is split off carries the four hydroxyl groups.

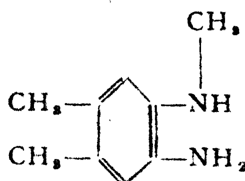
(*d*) Lumi-flavin, on boiling with aqueous alkali, gives rise to urea and a keto-carboxylic acid (A).



$$C_{13}H_{12}O_2N_4 + 2H_2O \longrightarrow H_2NCO{\cdot}NH_2 + \underset{(A)}{C_{12}H_{12}O_3N_2}$$

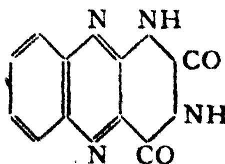
The above change involves the addition of two molecules of water and hence it is suggested that a ring containing two *CO* groups and one NH group, is decomposed; one of the *CO* groups is eliminated as urea and the other appears as a carboxyl group in A.

(*e*) The compound A, loses CO_2 on heating and gives a compound with the composition $C_{11}H_{12}ON_2$. The latter exhibits the typical behaviour of a lactam; the lactam on alkaline hydrolysis, gives 1.2-dimethyl-4-amino-5-methyl amino-benzene:

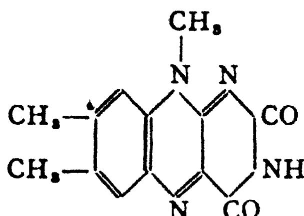


It gives a bluish-green colouration with $FeCl_3$ which according to Noelting, is characteristic of *p*-*p*′ disubstituted-o-phenylene-diamines.

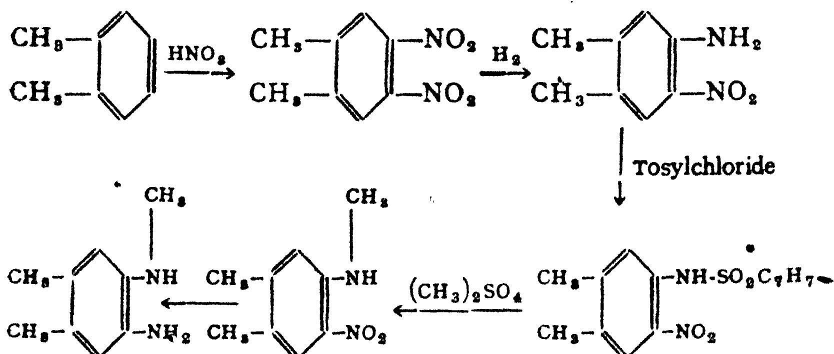
(*f*) Lastly, lumi-flavin resembles very closely the alloxazines in their general properties and reactions and the alloxazines are represented by the formula:



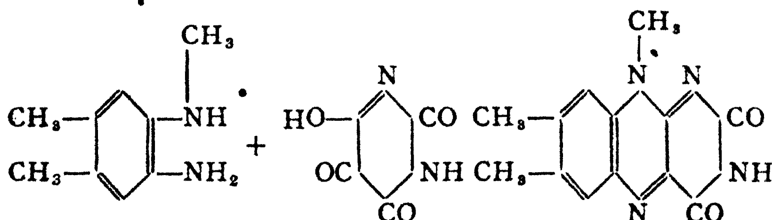
On the basis of the above evidence, lumi-flavin is assigned the structure:



The above structure is confirmed by a synthesis by Kuhn. The essential steps in the synthesis are:

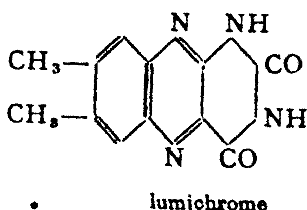


The last compound is condensed with alloxan in warm aqueous solution to give lumi-flavin.



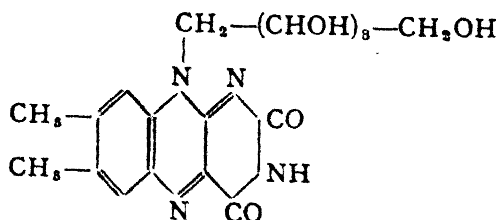
These results therefore indicate that the vitamin contains the lumi-flavin system, with a hydroxylated side-chain in place of the methyl group on the N-atom and the hydroxylated side-chain containing five

C atoms is probably derived from the pentose, ribose. The presence of the lumi-flavin system is further corroborated by the discovery of lumichrome by Karrer. Karrer found that ribo-flavin when irradiated in dilute methanolic solution (neutral) is decomposed and gives lumichrome $C_{12}H_{10}O_2N_4$. It resembles lumi-flavin very closely, but differs from it in lacking the N-CH_3 group. Its structure was finally established by a synthesis in which 4–5 diamino–o–xylene is condensed with allaxan.



lumichrome

On the basis of the above mentioned evidence, vitamin B_2 or ribo–flavin has been assigned the formula :



The exact nature of the side-chain was then established as follows: The side-chain contains three asymmetric C atoms and hence there are eight possible structures for the side-chain. The *L*-arabityl derivative was prepared and found to have very little vitamin activity, while the corresponding *D*-ribityl derivative was found to be identical with the natural vitamin B_2, in all its properties, including its specific physiological property. Hence the side-chain is related to *D*-ribose; the latter is a pentose widely distributed in nucleic acids.

Synthesis: A complete synthesis of the vitamin has been achieved by Kuhn and Karrer. It involves the following steps :

$$(CH_3)_2C_6H_3—NH_2 + OHC—(CHOH)_3—CH_2OH$$

↓ ribose, H_2 (catalytic)

$$(CH_3)_2C_6H_3—NH—CH_2—(CHOH)_3—CH_2OH$$

↓ $C_6H_5N_2Cl$

$$(CH_3)_2C_6H_2(—NH—CH_2—(CHOH)_3—CH_2OH)(—N{=}N—C_6H_5)$$

↓ $Na_2S_2O_4$

$$(CH_3)_2C_6H_2(—NH—CH_2—(CHOH)_3—CH_2OH)(—NH_2)$$

↓ alloxan in acetic acid containing boric acid

$$CH_2—(CHOH)_3—CH_2OH$$

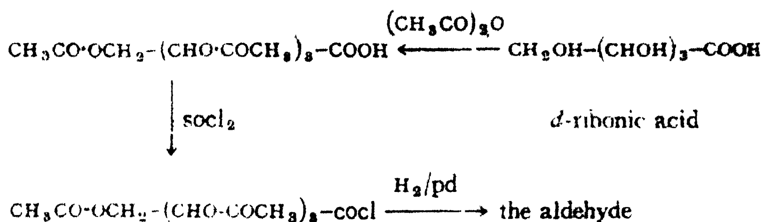
(isoalloxazine ring: CH_3, CH_3 on benzene ring; N, N, CO, NH, N, CO)

This method gives fairly good yields of ribo-flavin. However the method is very expensive as *D*-ribose is not readily available. Recently, a new method has been developed by Tishler which involves the following steps :

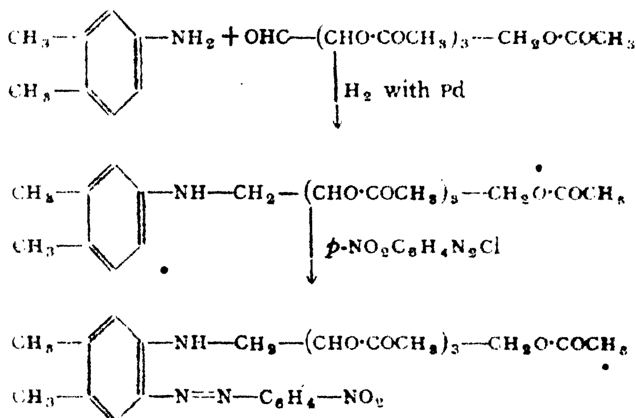
1. $HOCH_2—(CHOH)_4—CHO \longrightarrow CH_2OH—(CHOH)_3—COOH$

d-glucose → *d*-arabonic acid

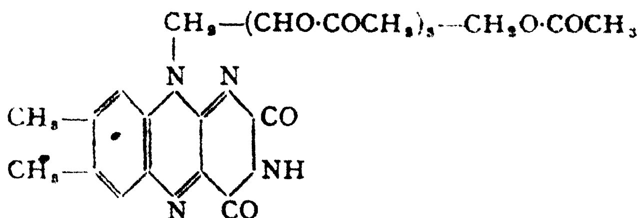
140° with pyridine



2.



The latter is condensed with barbituric acid to give tetra-acetyl-ribo-flavin :



which on mild alkaline hydrolysis gives the vitamin B_2.

The above method possesses the following advantages: (i) barbituric acid is more readily available and less expensive than alloxan, (ii) the ribose residue is obtained from the relatively cheap and readily accessible *D*-glucose.

Pantothenic acid

R. Williams and co-workers showed that extracts of different tissue material contained a substance which stimulated the growth of yeast. Later on, it was found that this growth principle was very widely distributed, but in small quantities; the yeast, egg-yolk, dried whey, rice bran, and liver extract constitute the more common and rich sources of the principle. It is called 'pantothenic acid' because of its almost universal presence.

Isolation : The above vitamin is best obtained from the concentrate of the alcohol soluble fraction of the liver extracts. The fraction is treated with water and subjected to adsorption with norite at pH 9·5; this removes the basic and other impurities. The acid is further purified by repeated extraction with absolute alcohol and precipitation of the remaining impurities with acetone or ether. Finally, the acid is obtained as the barium salt from the aqueous solution by using baryta. It is a pale-yellow* oil; it is *d*-rotatory; it forms micro-crystalline calcium and barium salts.

Constitution : The constitution of this acid was established by the brilliant and arduous researches of Williams and his co-workers. It is one of those rare cases, where the structure was established chiefly by the study of the decomposition products of a compound, even before its molecular composition was known.

(*a*) The molecular weight is found to be between 150-200.

(*b*) It behaves as a mono-carboxylic acid, forming salts and esters.

(*c*) As indicated by its ultra-violet absorption spectrum, it is aliphatic in nature and contains no double bonds.

(*d*) It contains nitrogen, but it is not an amide as hydrolysis produces no ammonia; it does not react with nitrous acid, thus showing the absence of amino group. But on hydrolysis with HCl, it gives β-alanine hydrochloride as one of the products. These results indicate that it is probably the peptide of β-alanine and another acid; hence it must be represented by R—CO—NH—CH_2—CH_2—COOH where R = the acid residue or the second half of the vitamin.

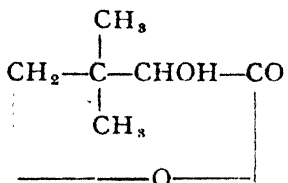
(e) The molecular composition of the second half is $C_6H_{10}O_3$.

(f) Titration values show that it contains no free—COOH group; however on heating with alkali, one equivalent of the latter is consumed. This indicates the presence of a lactone ring; the relatively great stability and the rate of lactonisation of the free acid show that it is the γ-lactone.

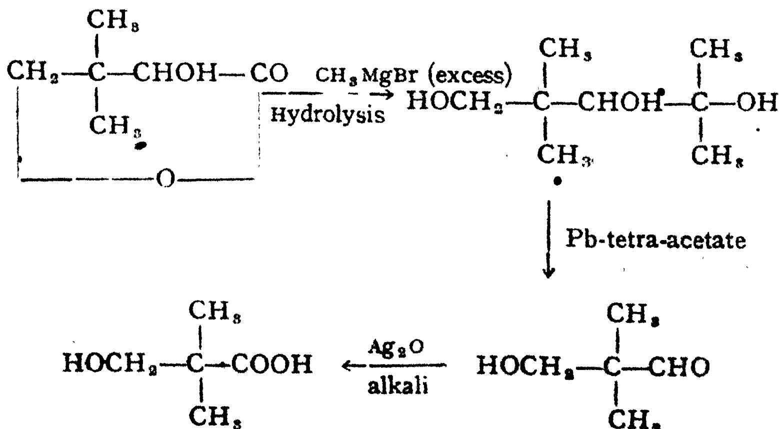
(g) It forms a mono-acetate and a 3-5-dinitro-benzoate, thus showing the presence of a hydroxyl group; that it is an α-hydroxy group, is indicated by a positive reaction with $FeCl_3$ and by the formation of CO with con. H_2SO_4. It also does not give the typical dehydration reaction characteristic of the β-hydroxy-acids.

(h) Lastly, Kuhn-Roth method of oxidation with CrO_3 gives indications that a gem-dimethyl group is present.

On the basis of the above evidence the second half of the vitamin is assigned the constitution :

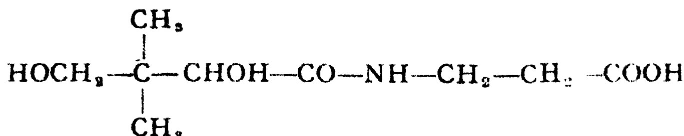


It is further confirmed by the following series of reaction :



The products corresponding to the different stages are isolated and identified thus proving the structure assigned to the lactone.

The constitution of the vitamin is then represented by:

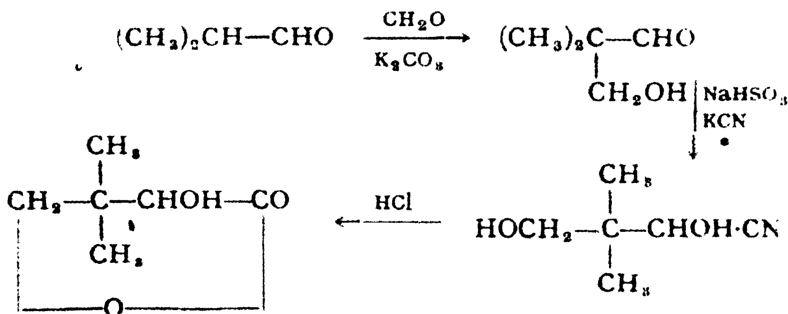


SYNTHESIS :—It involves the syntheses of (*i*) γ-butyrolactone and (*ii*) β-alanine; and condensing the two parts to give the vitamin

1. β-Alanine is obtained in good yields (75%) by the catalytic hydrogenation of the K-salt of cyanacetic acid in presence of NH_3 and methanol at 80° and under a pressure of 130 atm.

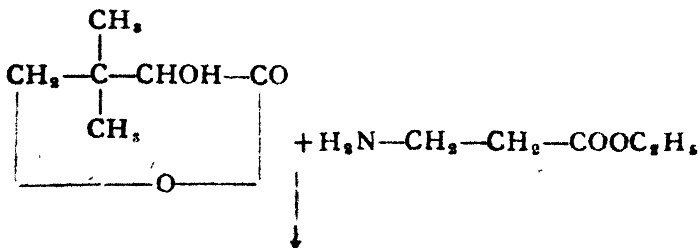


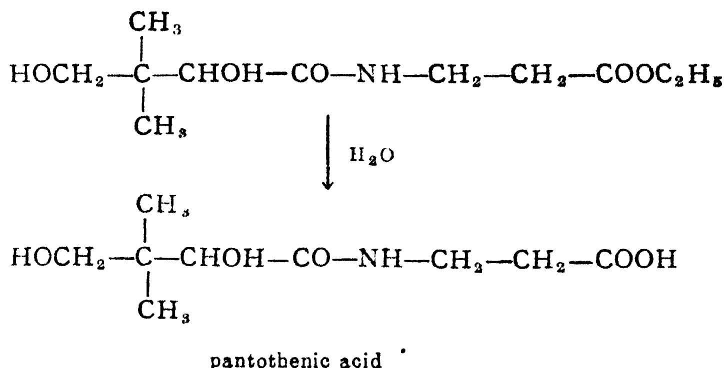
2. The γ-butyrolactone is obtained conveniently starting from iso-butyric aldehyde, according to the following series of reactions:



α-hydroxy-ββ-dimethyl-γ-butyro-lactone

3. The lactone thus obtained is condensed with the ethyl ester of β-alanine to give a compound which on hydrolysis gives the vitamin:





pantothenic acid

In a recent synthesis, the crystalline Na-salt of pantothenic acid is obtained by heating the dry lactone with the Na-salt of β-alanine. The product is crystallised from isopropyl alcohol. It is the purest form of the acid; it melts at 122°.

Vitamin B_6 or Pyridoxin

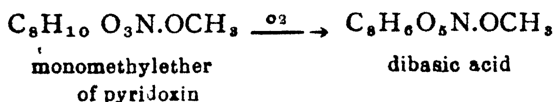
Occurrence:—This vitamin is widely distributed in plants and animals. Rice-bran and molasses constitute important sources; it is also very plentiful in yeast.

Isolation:—An aqueous extract of rice-bran is repeatedly extracted with ether at pH 7.5. It is then transferred to dilute H_2SO_4 and the solution concentrated to a small bulk; the pH is adjusted to 7.5 and the solution is treated with $AgNO_2$. All the impurities are quantitatively precipitated down and are removed by filtration. The filtrate is then acidified to pH 2.0 and the vitamin adsorbed on Fuller's earth. It is then eluted with baryta; the eluate is treated with calculated amounts of H_2SO_4 and $BaCl_2$. The inorganic salts are removed by filtration and the vitamin obtained as the hydrochloride, which is precipitated by the addition of acetone, ether etc. The hydrochloride crystallises from alcohol; m. p. 209°.

CONSTITUTION :—The constitution is established both by analytical and synthetic evidence.

(*a*) The molecular composition is $C_8H_{11}O_3N$.

(*b*) It forms a triacetate which can be distilled in vacuo; therefore, three OH groups are indicated; this is further confirmed by the Zerewitinoff's method. The vitamin gives a colour reaction with $FeCl_3$ and with diazo-methane a mono-methyl ether is formed thus showing the presence of a phenolic OH; the monomethyl ether, on oxidation with barium permanganate, gives a dibasic acid.



These results indicate the presence of two CH_2OH groups (4H atoms are replaced by two oxygen atoms).

(*c*) On oxidation with CrO_3 (Kuhn-Roth method.) one molecule of acetic acid is formed from one molecule of the vitamin: hence one C-methyl group is indicated.

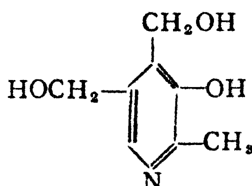
(*d*) From (*b*) and (*c*), it follows that of the eight C atoms, two are present as CH_2OH groups and one as—CH_3; the remaining five may therefore be present in combination with N, as the pyridine ring. This is confirmed by the following reactions; (*i*) pyridoxin does not react with nitrous acid; (*ii*) with $FeCl_3$, a deep reddish-brown colour is formed, which is characteristic of β-hydroxy-pyridine; (*iii*) pyridoxin gives a stable hydrochloride.

(*e*) The dibasic acid discussed under (*b*), does not give any colour reaction with aqueous $FeSO_4$ solution; this indicates that neither of the two carboxyl groups, is in α-position to the nitrogen. Further, on fusion with resorcinol, the dibasic acid forms a fluorescent dye thus showing that the two COOH groups are in ortho-positions to each other. Hence they must be present in β and γ positions. The two primary alcoholic groups are thus present in β and γ positions.

(*f*) The phenolic OH group is present in β position; see (*d*) above.

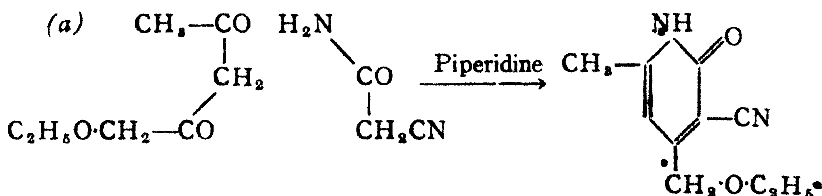
(*g*) The exact position of the methyl group is indicated by the specific test which the vitamin gives with dichloro-quinone-chlorimide. It is a test for the presence of a free nuclear position para to a phenolic OH group. Hence the CH_3 group must be present in the α position which is *not* para to the β-hydroxyl group.

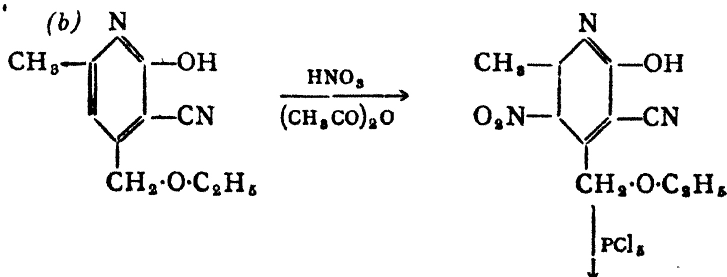
The above evidence therefore clearly shows that pyridoxin is:

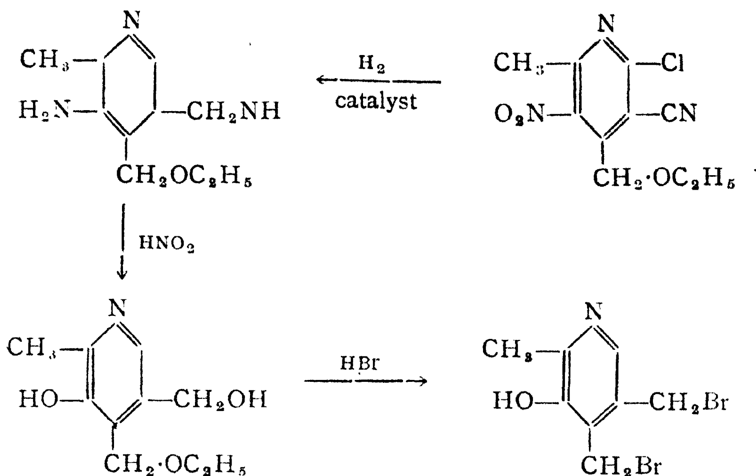


SYNTHESIS OF PYRIDOXIN: There are two methods by which the vitamin has been obtained: one is based on the direct building up of the pyridine nucleus and the other based on the degradation of a suitable quinoline derivative.

First method:—The essential steps are:—

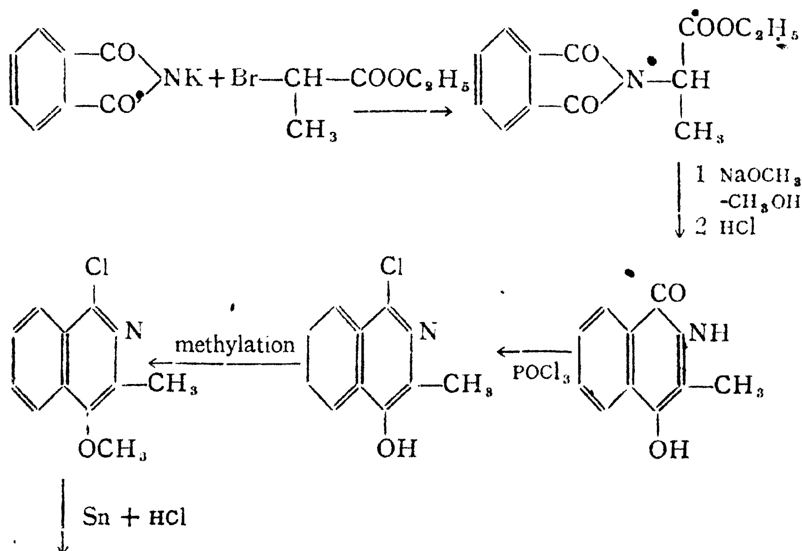


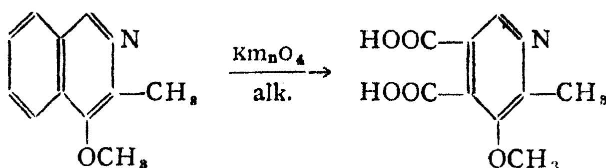




The di-bromide on treatment with silver acetate gives the vitamin; the bromine atoms are replaced by hydroxyl groups.

Second method :—In this method the suitable quinoline derivative is first synthesised; the steps involved are :





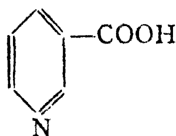
The dibasic acid is then converted into the diamide; the latter is then dehydrated to the di-nitrile; on catalytic reduction, the CN groups are changed into CH_2NH_2 groups which are subsequently converted into primary alcoholic groups by the action of HNO_2. The compound thus obtained is the methyl ether of pyridoxin; it is converted into the latter by treatment with HBr and by subsequent reaction with silver acetate.

Nicotinic acid or Niacin

Occurrence :— Nicotinic acid and its amide occur widely in small quantities, in many plants and animal tissues. Cereals like wheat, soya-bean, oats and rice also contain small amounts of nicotinic acid; yeast constitutes one of the richest natural sources.

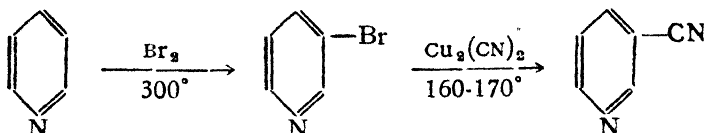
It is not isolated from any natural source, but is obtained synthetically by different methods described later on. It is a crystalline compound, m. p. 234°. The acid and the amide are known as the antipellagra factor. It is very effective in curing pellagra in human beings and the condition known as black tongue in dogs.

Constitution :— The molecular composition of the acid is $C_6H_5O_2N$. It forms a mono-sodium salts thus indicating, that it is a mono-carboxylic acid; on distillation with lime, the acid gives pyridine. Hence it is a carboxylic derivative of pyridine. That it is a β-carboxylic derivative of pyridine, is indicated from its formation from β-picoline by oxidation with chromic acid. Hence nicotinic acids is :



SYNTHESIS :—There are several methods by which nicotinic acid is prepared on a large scale. In one method, nicotine, the alkaloid present in tobacco leaves is oxidised with nitric acid (27%) chromic acid or with permanganate.

Another method starts from pyridine and the steps involved are :—



The nitrile is purified by vacuum distillation ; on hydrolysis with alcoholic NaOH and subsequent acidification (HCl and Na-acetate) nicotinic acid is obtained. If necessary, the amide is obtained directly from the nitrile by hydrolysing it with concentrated aqueous ammonia at 108-109°.

Recently, methods have been developed for obtaining nicotinic acid, which are based on oxidation of quinoline or a quinoline derivative. e. g. hydroxy-quinoline ; the quinolinic acid thus obtained is then decarboxylated to give nicotinic acid.

Quinoline is now simultaneously oxidised and decarboxylated to give nicotinic acid, by treating it with con. H_2SO_4 and SeO_2 at 300°.

Vitamin C

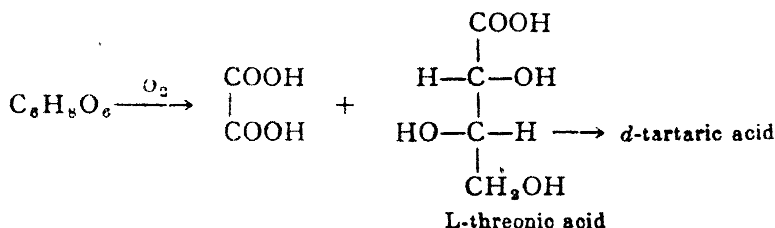
Occurrence :—It is present in fruits like lemons, oranges, black currants and also in green vegetables, *e.g.*, cabbage, beans and tomatoes. It is the most abundant of the known vitamins. This vitamin, in solution, is extremely sensitive to oxygen and heat. It is the most easily destroyed of all known vitamins. It has an anti-scorbutic activity and is *d*-rotatory (+24°); configurationally, it belongs to the L-family and hence its name 'L-ascorbic acid'. Its deficiency in the diet causes the disease called scurvy, brittleness of bones and greater susceptibility to disease.

Isolation :—It was first isolated in the crystalline form by Szent Gyorgyi from the adrenal cortex and later on, from the Hungarian pepper and also from the juice of the fully ripe capsicum. It is now

more easily obtained from glucose by a synthetic method. It was the first vitamin to be synthesised. It is a crystalline compound, m.p. 189-192°. It is soluble in water, alcohol, and glycerol; it is an acid in aqueous solution; it is a powerful reducing agent.

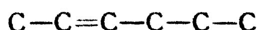
Constitution :—Its molecular composition is $C_6H_8O_6$. It gives the following reactions :—

(*a*) NATURE OF THE CARBON SKELETION :—(Results of oxidation and ozonolysis). On oxidation with acid $KMnO_4$ L-ascorbic acid gives oxalic acid and L-threonic acid (*i.e.* tri-hydroxy-butyric acid), the latter on further oxidation, gives *d*-tartaric acid.



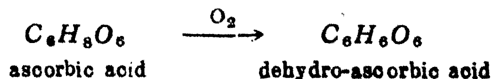
Hence the molecule must contain a four carbon system (butyric acid) separated from a two carbon system (oxalic acid) by a double bond.

The presence of the double bond is further confirmed by the isolation of a mono-ozonide of the composition $C_6H_8O_6.O_3$. The carbon chain in the molecule may be represented by :



Its composition (C_6) and solubility in water suggest a close relation to hexoses. This is further confirmed by the quantitative formation of furfuraldehyde, on boiling the vitamin with con. HCl. Also, acetone condenses with the vitamin, as it does with sugars, to give a mono-acetone derivative.

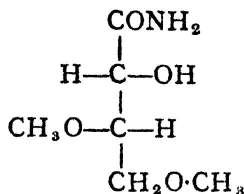
(*b*) THE NATURE OF OXYGEN ATOMS :—This is established by studies in mild oxidation and methylation. L-Ascorbic acid is rapidly oxidised by iodine in alkaline solution to form a compound $C_6H_6O_6$.



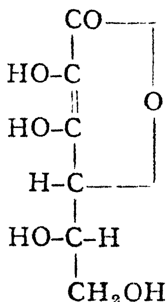
The oxidation is reversible and the product is neutral. As the oxidation by alkaline iodine solution involves the elimination of two hydrogen atoms with the formation of a neutral compound, the presence of two *enolic hydroxyl* groups is indicated. The methylation with diazomethane gives a dimethyl derivative, and diazomethane is a specific reagent for methylation of enolic groups. These facts further confirm the presence of enolic groups. This is also in agreement with the weak acidity of the molecule and its violet colour reaction with $FeCl_3$ (enolic compounds give these reactions). The dimethyl derivative further gives a di-derivative with *p*-nitro-benzoyl chloride which further indicates the presence of two more *OH* groups. The presence of four hydroxyl groups in all, in the molecule of the vitamin is confirmed by the formation of a tetramethyl derivative, on methylation of the vitamin with Ag_2O and methyl iodide.

The dimethyl either of ascorbic acid, on oxidation with lead tetra-acetate yields formaldehyde as one of the products of oxidation. These results show that there are two vicinal alcoholic hydroxyl groups and further one of them is a primary alcoholic group. (see p. 18).

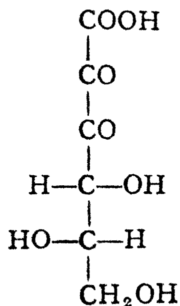
Lastly, the dehydro-ascorbic acid $C_6H_6O_6$ is a lactone; it is rapidly hydrolysed and the rate of hydrolysis compares favourably with that of the hydrolysis of γ-lactones. Therefore, the oxidation product of the vitamin contains a γ-lactone ring; it is also possible that the vitamin itself contains such a lactone ring. Hirst has provided a direct proof for the absence of COOH group in the vitamin. He showed that the dimethyl ether derivative obtained by the action of CH_2N_2 on the vitamin is neutral dissolved in alkali without the splitting of a methyl group; a —$COOCH_3$ under these conditions would give CH_3OH. Hence no methyl ester group was present; this indicates the absence of a COOH group in the vitamin. The behaviour of the above derivative on the other hand resembles that of a lactone; the exact nature of the lactone ring was experimentally established by methylation studies. The tetramethyl derivative obtained by the action of CH_3I and Ag_2O on the vitamin is ozonised and the product treated with ammonia. Oxamide and another amide are obtained. The latter reacts with Na-hypochlorite to form Na-isocyanate (Weerman's reaction for α-hydroxy amides) and is identified as 3·4 dimethyl-L-threonamide.



The hydroxyl group in ∝-position to the $CONH_2$ group shows that this carbon atom must have been involved in the lactonisation. But it is the third carbon atom from the primary alcoholic group. Hence the lactone is a-γ-one. This is in good agreement with the formation of furfuraldehyde, on boiling with HCl. Further, as—CH_2OCH_3 forms the end of the four carbon system, the lactone group—C = O, must form the end of the two carbon system. Hence, the complete structural formula for *l*-ascorbic acid would be, it is the enolic form of 2-ketol-gulono-lactone :



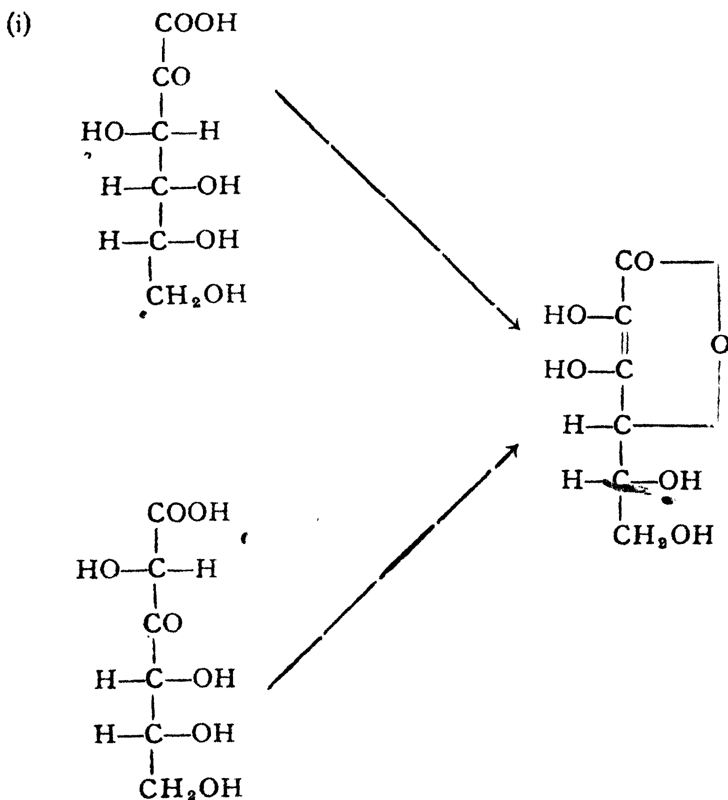
The exact *configuration* of the two C_4 and C_5 atoms was derived from (*a*) its relation to L-threonic acid, the product of ozonolysis or of oxidation of the molecule and (*b*) that of the oxidation product $C_6H_8O_7$ which was shown to be 2-3 diketo-L-gulonic acid :—

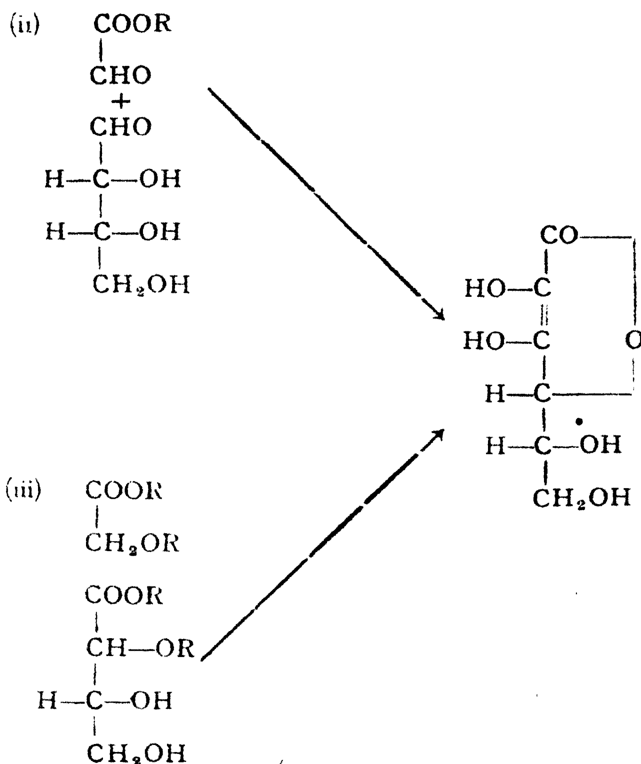


The results show that the vitamin is configurationally related to L-gulose. The latter on oxidation gives L-threonic acid. The oxidation of L-ascorbic acid finally to tartaric acid also confirms the above configuration; C_4 is positive as the acid is dextrorotatory and C_5 is therefore negative.

SYNTHESIS:—The above structure is confirmed by a synthesis. The synthesis has been accomplished by four different methods based on different principles. The methods are: (*i*) isomerisation and lactonisation of 2 or 3-keto hexonic acids, (*ii*) benzoin condensation between two different aldehydes of lower molecular weight and (*iii*) ester condensation of α-oxy-acids. Schematically:

(i)

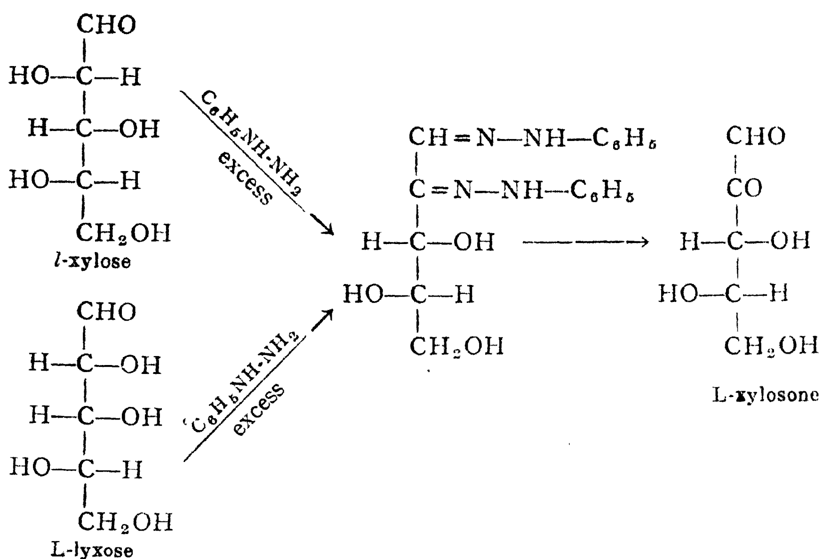




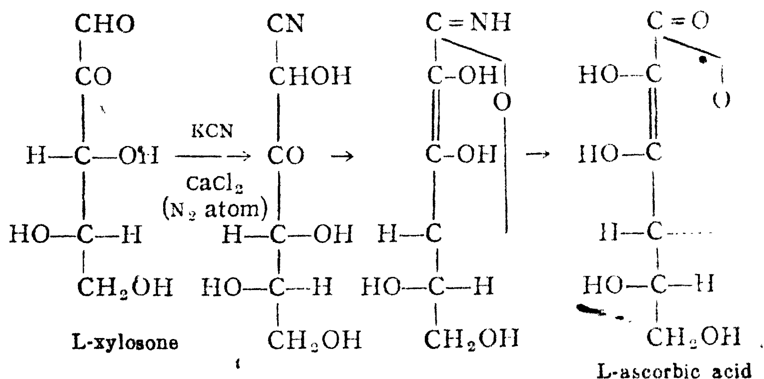
The first synthesis reported was that by Haworth and Hirst; it is based on the isomerisation and lactonisation of 3-keto-hexonic acid obtained by the osone-hydrogen cyanide method. The osone used was the L-xylosone. L-Xylosone was obtained from both the pento-aldoses: L-xylose and L-lyxose; both these sugars are not readily available in sufficient quantities and hence they were obtained from D-galactose by a tedious process (D-galactose → D-galacturanic acid → D-galactonic and → L-lyxose).

The different steps involved in the ascorbic acid synthesis are:

(*a*) Conversion of L-xylose or L-lyxose into L-xylosone:

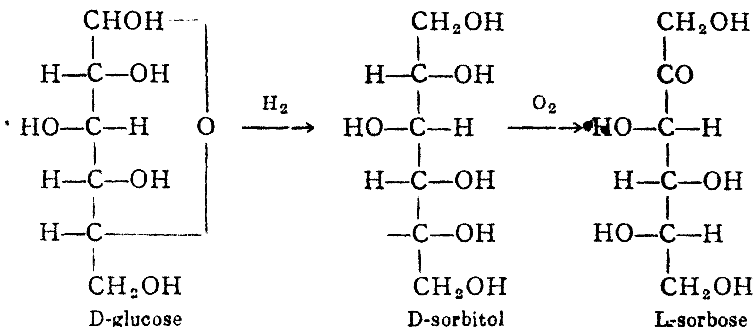


(*b*) Conversion of L-xylosone into L-ascorbic acid.



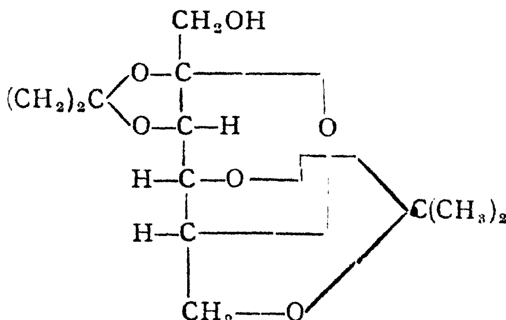
The use of KCN in presence of $CaCl_2$ as a source of HCN, shortens the reaction time from days to hours. The cyclic-imino compound has been isolated, but its isolation is not necessary. On hydrolysis with 8% HCl at 40 to 50° 26 hours, it is quantitatively converted into L-ascorbic acid, identical with the natural product. However the most difficult part of the synthesis is the preparation of the osone.

Another important synthesis of the vitamin is due to Reichstein and Haworth. It is based on the same principle as in the synthesis mentioned above. The starting point, however, for this synthesis is D-glucose; it is reduced to D-sorbitol by catalytic reduction using a special catalyst consisting of Ni, Co and Cr; D-sorbitol is then oxidised to L-sorbose by the action of *Aceto bacter Suboxydans* which is capable of bringing about the oxidation in 3–5 days; the *Bacterium xylinum* or Bertrand's bacterium from the juice of the mountain-ash-berries requires six weeks.

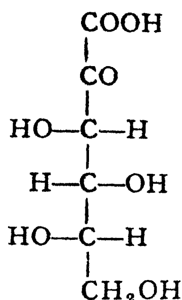


L-Sorbose is then converted into 2-keto-L-gulonic acid as follows.

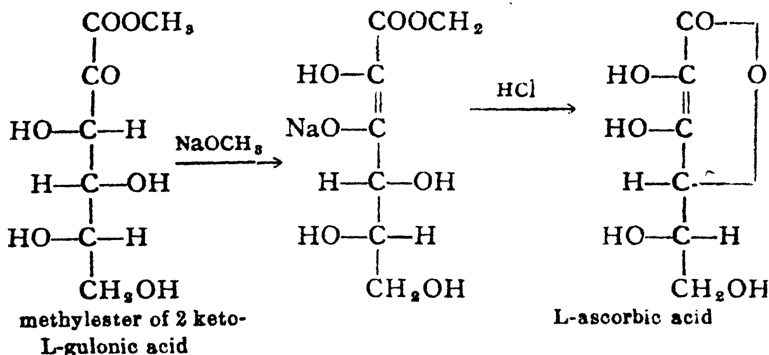
L–sorbose is condensed with acetone to protect all the hydroxyl groups except the primary one in position 1, thus giving the 2, 3, 4, 6 diacetone derivative of L–sorbose :



The diacetone sorbose is oxidised with alkaline $KMnO_4$ to give the corresponding 2, 3, 4, 6 diacetone derivative of 2–keto-L-gulonic acid. On warming in water containing a small amount of acid, the latter yields the free 2-keto-L-gulonic acid.



The isomerisation and lactonisation of the above acid to yield ascorbic acid, are effected as follows: the free acid is not isolated but immediately converted into the methyl ester with CH_3OH and HCl gas: the ester is heated with $NaOCH_3$, when the Na-salt of the L-ascorbic acid is formed. Acidification with HCl gives the free acid, L-ascorbic acid.



methylester of 2 keto-L-gulonic acid

L-ascorbic acid

Technically the vitamin is now obtained by this method.

Recently a new process for the production of the vitamin has been developed in U. S. A. The pectic substances–the by–products of the sugar beet industry constitute the source material. The pectic substances are converted into D–galacturonic acid by the action of pectinases. The ceronic acid is then reduced by Ni and H_2 to L–galactonic acid. The latter on heating with oxalic acid gives the lactone which is oxidised with $NaClO_3$ in presence of V_2O_5, to 2–keto-L galactonic acid. The latter is then converted into 1 ascorbic acid by the same process as is used in the case of 2–keto-L gallonic acid discussed above.

Biotin or Vitamin H

Occurrence :—This vitamin occurs in small amounts in yeast, egg-yolk, liver, kidney and milk. Rice and wheat brans also contain considerable amounts of this vitamin. It is usually present in a form which is insoluble both in fats and in water, but is hydrolysed and rendered soluble.

Isolation :—A convenient source is milk concentrates. The vitamin present is converted into the methyl ester. The latter is then adsorbed from a chloroform solution on activated alumina ; it is then eluted with methanol-acetone mixture. The eluate yields the methyl ester of the vitamin in a crystalline form ; purification finally by vacuum sublimation, gives the ester m. p. 166°. On saponification with alkali in the cold and subsequent acidification, the vitamin is obtained in the form of long needles. m. p. 232 (dec.). It is *d*-rotatory.

Constitution :—The molecular formula of biotin is $C_{10}H_{16}O_3N_2S$. The structure is based on the following analytical evidence :

(*a*) It forms readily a mono-methyl ester thus indicating the presence of a COOH ; this is confirmed by the results of titration with alkali.

(*b*) It can neither be acylated nor alkylated, which shows the absence of any hydroxyl group.

(*c*) It does not react with any carbonyl reagent. Hence aldehydic or ketonic groups are absent.

(*d*) No nitrogen is formed, when the vitamin is made to react with HNO_2; also the ninhydrin reaction is negative. Hence the vitamin is not an *amino acid.*

(*e*) On heating with $Ba(OH)_2$ at 140° for several hours, biotin is decomposed into a compound A with the loss of one C and one O atom :



$$\underset{\text{biotin}}{C_{10}H_{16}O_3N_2S} \longrightarrow \underset{\text{A}}{C_6H_{18}O_2N_2S}$$

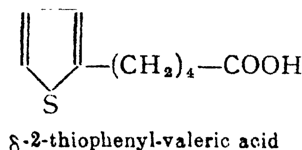
The compound A is shown to be a monobasic acid, containing two free NH_2 groups (a dibenzoyl derivative is obtained). Its formation from the vitamin with the loss of a *CO* group, suggests

that the vitamin is a cyclic urea derivative. This is confirmed by the partial synthesis of the vitamin from compound A and $COCl_2$. Further, the diamino-compound is condensed with phenanthrene-quinone in acetic acid to give a quinoxaline derivative. These results show that it is 1 : 2 diamine and therefore the urea unit in the vitamin is present as a 5-membered ring.

The structure of compound A is further elucidated as follows :

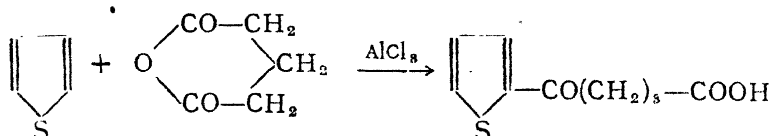
(*i*) On oxidation with nitric acid, A yields adipic acid. But the oxidation of the amine obtained from methyl ester of the vitamin by the Curtius degradation, gives no adipic acid. Hence it follows that one of the COOH groups of adipic acid, must be the COOH group originally present in the vitamin.

(*ii*) A is methylated with $(CH_3)_2SO_4$ and potash, and the product is heated with HCl, when δ-2-thiophenyl-valeric acid is obtained.

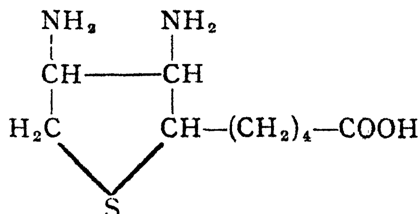


δ-2-thiophenyl-valeric acid

The identity of this product is established by a direct synthesis.



The latter on reduction with amalgamated zinc gives the compound identical with one obtained from compound A. Hence compound A must be represented by :



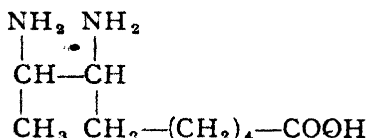
The above structure is further confirmed by the following additional evidence.

The presence of the sulphur atom as a thio-ether is indicated by the formation of a stable sulphone on treatment with H_2O_2 in glacial acetic acid.

The above structure for A is independently proved by a study of dethio-biotin. The latter is obtained when the methyl ester of biotin is reduced with Raney Ni, in boiling ethanol.

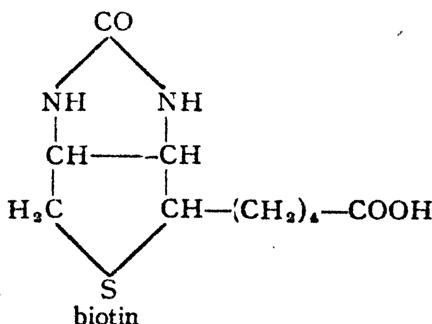


Dethio-biotin contains the same number of C, N and O atoms; further on heating with $Ba(OH)_2$ at 140°, dethio-biotin gives 7-8-di-amino-nonoic acid:



The above structure is proved by a synthesis from ω-bromo-hexoic ester and mono-sodio-aceto-acetic ester and subsequent amination.

The vitamin is obviously the cyclic urea derivative of A and therefore is represented by:



biotin

SYNTHESIS :—The synthesis involves the preparation of two key-compounds: (*i*) 4-benzamido-3-keto-tetrahydro-thiophene (A) and (*ii*) methyl-γ-formyl-butyrate (B).

(*i*) The thiophene derivative has been obtained according to the following procedure :

$$HS{\cdot}CH_2{-}\underset{\displaystyle NH_2}{\underset{|}{C}}H{-}COOH + ClCH_2{\cdot}COOH \xrightarrow[NaOH]{} \begin{matrix} NH_2 \\ | \\ CH{-}COOH \\ | \\ CH_2 \quad CH_2{\cdot}COOH \\ \diagdown \; \diagup \\ S \end{matrix}$$

$$\Big\downarrow \; \begin{matrix} 1\ C_6H_5COCl \\ 2\ CH_3OH + H_2SO_4 \end{matrix}$$

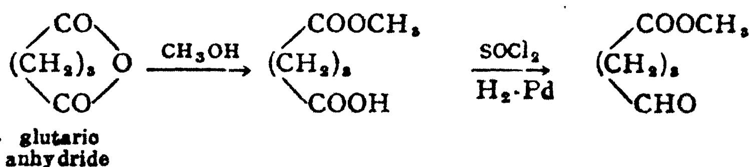
$$\begin{matrix} NH{-\!-\!-}COC_6H_5 \\ | \\ CH{-\!-\!-}C{-}ONa \\ | \qquad\quad \| \\ CH_2 \qquad C{-}COOCH_3 \\ \diagdown \; \diagup \\ S \end{matrix} \xleftarrow{NaOCH_3} \begin{matrix} NH{-\!-\!-}COC_6H_5 \\ | \\ CH{-}COOCH_3 \\ | \\ CH_2 \quad CH_2{-}COOCH_3 \\ \diagdown \; \diagup \\ S \end{matrix}$$

$$\begin{matrix} CH_3COOH \\ HCl \end{matrix} \Big\downarrow \begin{matrix} \text{Hydrolysis} \\ \text{decarboxylation} \end{matrix}$$

$$\begin{matrix} NH{-}COC_6H_5 \\ | \\ CH{-\!-\!-}CO \\ | \qquad\quad | \\ CH_2 \qquad CH_2 \\ \diagdown \; \diagup \\ S \end{matrix}$$

A

(*ii*) Methyl-γ-formyl-butyrate has been prepared by the following procedure :



(*iii*) A and B are then condensed together in presence of pyridine and acetic acid and the product formed, is subjected to a series of reactions to give the vitamin : biotin.

$NH—COC_6H_5$
$CH——CO$
$CH_2 \quad CH_2$ + $OHC·(CH_2)_3·COOCH_3$ → $NH—COC_6H_5$ / $CH——CO$ / $CH_2 \quad C=CH—(CH_2)_3COOCH_3$
S S

↓

$NH—COC_6H_5$
$CH——C=NOH$
$CH_2 \quad C=CH·(CH_2)_3—COOCH_3$
S

Zn, $(CH_3CO)_2O$

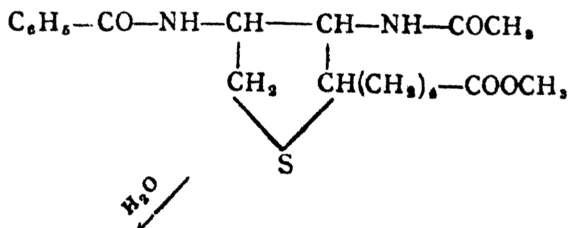
$NH—COC_6H_5$
$CH——CH—NH—COCH_3$
$CH_2 \quad C=CH—(CH_2)_3·COOCH_3$
S

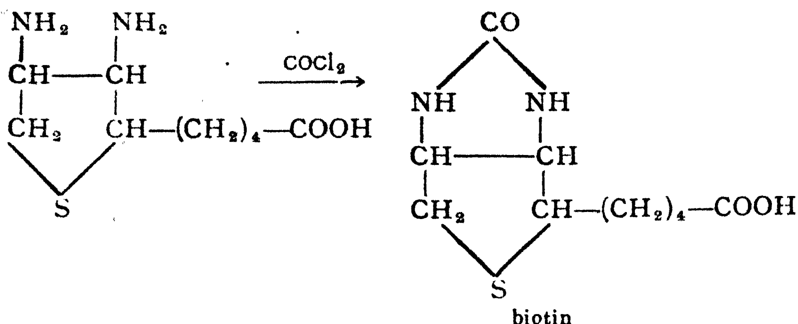
C

$NH—COC_6H_5$
$CH——C—NH—COCH_3$
$CH_2 \quad C—(CH_2)_4—COOCH_3$
S

D

Both the compounds C and D on reduction with H_2 and Pd, give the same compound which is then converted into biotin.

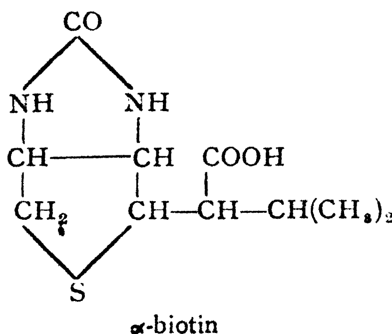




biotin

The synthetic product is *dl* biotin and is half as active as the natural *d*-isomer. The *d*-isomer is obtained from the synthetic product by resolution with *l*-arginine.

Recently, Kögl and E. J. ten Ham have reported that the biotin from egg-yolk is not identical with that from liver. Kögl calls the liver-vitamin α-biotin, and has proposed the structure :



α-biotin

This isomer has the same physiological activity as the β-isomer.

Vitamin E

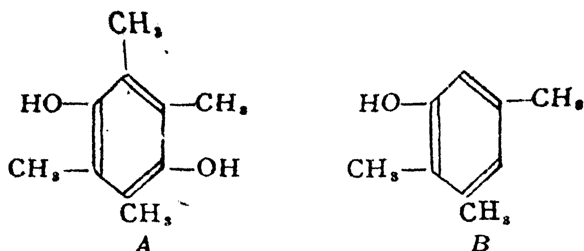
Occurrence:—This vitamin occurs in leafy vegetables like spinach and lettuce. Wheat-germ oil is one of the richest sources of this vitamin ; it is also present in cotton-seed oil. It is very susceptible to oxidation and rancidity completely destroys the potency.

Isolation:—The wheat-germ oil is saponified in an atmosphere of N_2 and the unsaponifiable portion is worked up for the vitamin. The unsaponifiable matter contains sterols which are removed by precipitation with digitonin. The remaining oil is the impure vitamin; it is then saturated with cyanic acid gas obtained by heating cyanuric acid. A crystalline allophanate m.p. 158° separates out; on hydrolysis, it gives a light-yellow oil which possesses the vitamin E activity, *i.e.* it can cure sterility in rats. It is called α-tocopherol. After the removal of the α-tocopherol-allophanate, the mother liquor gives another crystalline compound m.p. 144°; on hydrolysis, it gives a compound possessing activity half of that of α-tocopherol; it is called β-tocopherol. The above procedure, however involves considerable loss of the vitamin, during the saponification stage. Hence Drummond and others have used the adsorption technique for the separation of the vitamin; it is thus adsorbed on alumina from a solution of the oil in light petroleum. The yields of the two tocopherols are greatly improved. Lastly a third allophanate m.p. 183° is isolated from cotton-seed oil (besides the α and β isomers mentioned above); on hydrolysis, it gives the γ-tocopherol with only one-third of the activity of the α-isomer. The tocopherols thus obtained are viscous oily liquids,, with a pale-yellow colour; they melt at about 0°. They dissolve in fats and other common organic solvents. They are very susceptible to oxidation and are completely destroyed by oxygen. The constitutional relationships of the three α, β and γ-tocopherol are discussed separately.

Constitution:—α-Tocopherol has the molar composition $C_{29}H_{50}O_2$. This suggests a relationship to the sterols (C_{28-29}) with which it is also associated. However, the study of the absorption spectra revealed that such a structural relationship does not exist. The constitution is based on the following evidence:

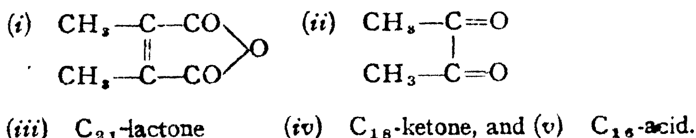
(*a*) It forms a monoacetate, and a mono-allophanate thus showing the presence of hydroxyl group; the phenolic character of this hydroxyl group is indicated by the observed shift of the absorption maximum towards shorter wave-lengths by about 20 mu, on esterification.

(*b*) Distillation of α-tocopherol in vacuum, gives duro-hydroquinone (A) while iso-pseudo-cumenol (B) is obtained by heating the vitamin with HI.



These results indicate the presence of a benzene nucleus carrying a hydroxyl and a few methyl groups. The second oxygen atom is probably present as an ether system *i. e.* chromane or furane rings. This is indicated by the appearance of one more hydroxyl group in *A* and the loss of one methyl group in the formation of *B*. The exact nature of the ring is revealed by the results of oxidation.

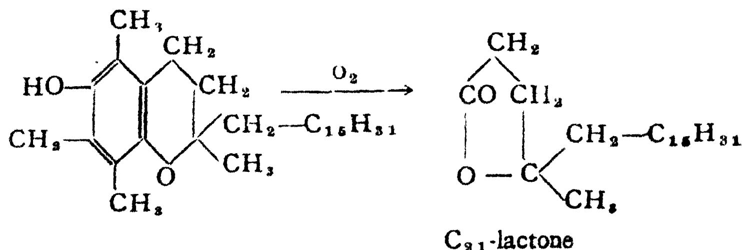
(*c*) When α-tocopherol is subjected to oxidative degradation with chromic acid, the following products are obtained :



(*iii*) C_{21}-lactone (*iv*) C_{18}-ketone, and (*v*) C_{16}-acid.

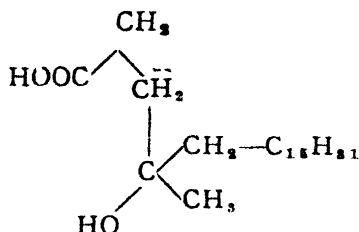
The C_{21} lactone gives a hydroxy acid which lactonises readily thus indicating that it is a γ hydroxy acid. The methyl ester of the hydroxy acid, cannot be oxidised to a keto-acid hence the OH group is tertiary. This is further confirmed by the observed difficulty of esterifying this hydroxyl group.

These results can only be satisfactorily accounted for, on the basis of a chroman structure.



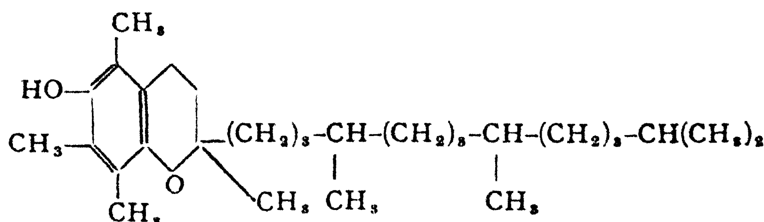
C_{21}-lactone

Such a C_{21} lactone can also give rise to a C_{18} ketone and a C_{16} acid which are among the products of oxidation. The corresponding hydroxy-acid will be :

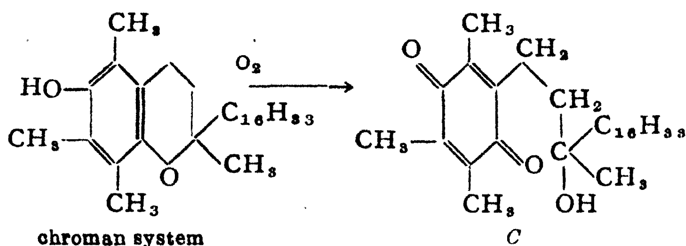


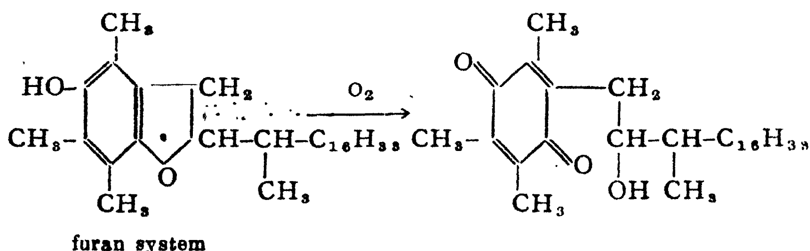
The nature of the side-chain ($C_{16}H_{31}$) is deduced from analogy to many natural products like the terpenes, which follow the isoprene rule. Thus three isoprene units are indicated in the side-chain. The C-methyl determination of the C_{16} acid actually indicated the presence of three methyl groups, on the basis of the above results, a phytol structure for the side-chain was first proposed and subsequently confirmed by an actual synthesis.

Hence α-tocopherol is assigned the following formula :



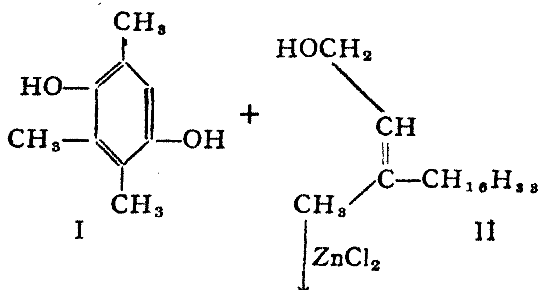
P. Karrer and collaborators have independently proved the existence of a chroman ring in the vitamin molecule, as follows : The vitamin on careful oxidation with $AgNO_3$ or $AuCl_3$, gives a yellow quinone (C) : the aliphatic hydroxyl group in the latter, shows on oxidation and esterification the typical behaviour of a tertiary hydroxyl group. The presence of a furan ring, leads under the conditions, to a hydroxy-quinone (D) containing a secondary alcoholic group.

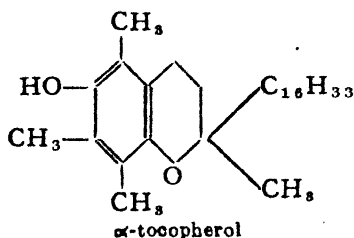




The furane system also cannot give rise to the C_{21} lactone; further, the hydroxy-acid that is possible with such a structure, will be a β-hydroxy acid that will give rise to an unsaturated acid on heating and a ketonic acid on oxidation.

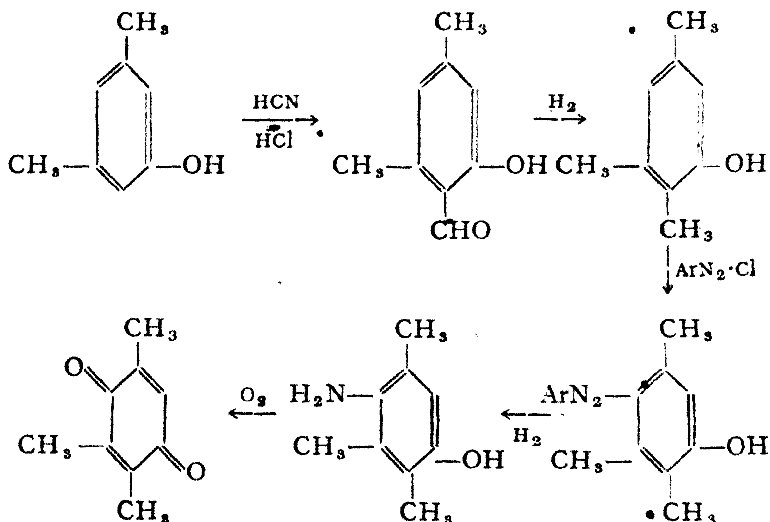
Synthesis : Karrer synthesised the vitamin by heating ψ cumoquinol (I) and phytyl bromide (II) in benzene solution in presence of $ZnCl_2$. Later on Todd showed that phytol can be used in place of the phytyl-bromide, with good results.





α-tocopherol

The ψ cumi-quinol (Cuminol) has been obtained by the following steps :



The last compound on reduction gives the ψ-cumi-quinol.

The above synthesis by Karrer and Todd suffers from two disadvantages : it does not decisively prove the existence of the chroman ring and secondly, phytol is not easily available and is very expensive. These disadvantages are eliminated in the recent synthesis by Smith and collaborators. It involves the following steps :

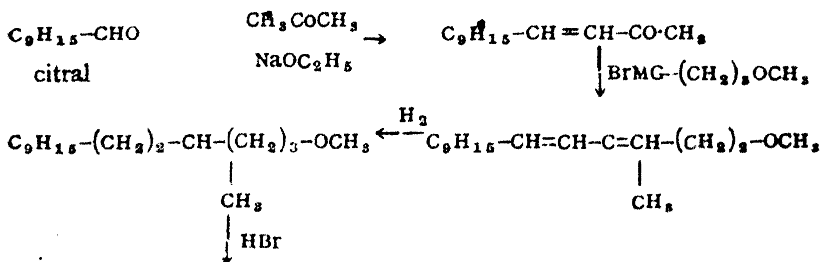
CH_3, CH_3O—, —MgBr, CH_3—, —OCH_3, CH_3 + CH_2——CH_2 (O) ⟶ CH_3O—, $-CH_2-CH_2OH$, CH_3—, $-OCH_3$, CH_3, CH_3

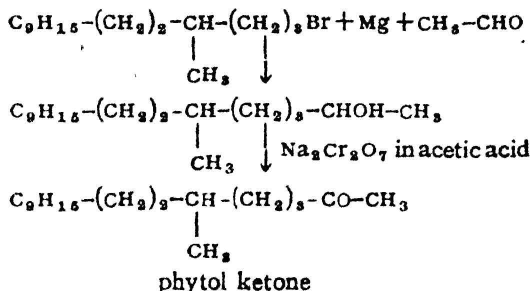
↓ 1. PBr_3 2. Mg

$C_{16}H_{33}$—CO—CH_3 (phytol ketone) + CH_3, CH_3O—, $-CH_2-CH_2MgBr$, CH_3—, —OCH_3, CH_3

CH_3, CH_3O-, CH_2-CH_2, CH_3-, $-OCH_3$, CH_3, C–$C_{16}H_{33}$, HO, CH_3 —HBr⟶ CH_3, HO–, CH_3-, O, $C_{16}H_{33}$, CH_3, CH_3

The phytol ketone used in the above synthesis, can be obtained from citral which is readily available. The essential steps are given below:

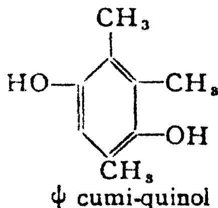




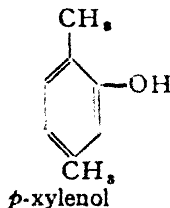
phytol ketone

β–Tocopherol: The constitution of this vitamin is based on the following evidence: (*a*) Its molecular composition is $C_{28}H_{48}O_2$ *i. e.* it contains one CH_2 group less than α-tocopherol.

(*b*) On distillation in high vacuum, trimethyl-hydroquinone *i. e.* ψ cumi-quinol is obtained, while on heating with HI, *p*-xylenol is formed.



ψ cumi-quinol



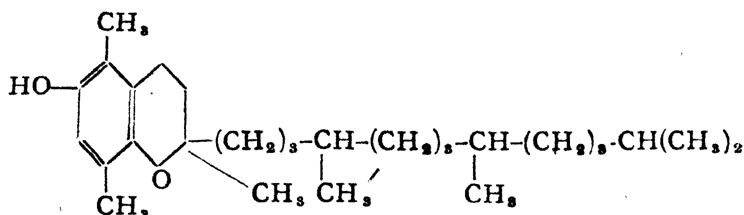
p-xylenol

These results indicate the presence of a benzene nucleus, carrying methyl and hydroxyl groups.

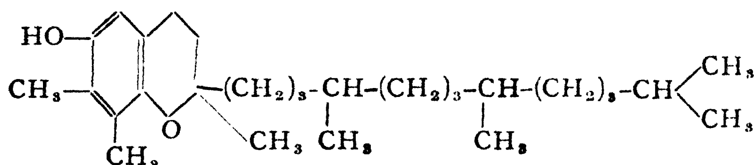
(*c*) It forms a monoester, thus indicating the presence of one hydroxyl group, which is revealed to be a phenolic one by the study of the absorption spectra of the ester.

(*d*) On chromic acid oxidation, the vitamin gives the same C_{21} ketone as the α isomer.

The above results thus show that β-tocopherol is the lower homologue of α–tocopherol with only two methyl groups in the benzene ring; further these two methyl groups are in para positions to each other. Hence β–tocopherol is :



γ-Tocopherol: It has the molecular composition $C_{28}H_{48}O_2$ and is thus isomeric with the β-isomer. On pyrolysis, it gives trimethyl-hydroquinone, the same compound as given by the β-isomer. In the β-isomer, the two methyl groups are in para positions to each other in the benzene nucleus; in the γ isomer, they may be ortho to each other: this is confirmed by the formation of dimethyl-maleic anhydride on chromic acid oxidation. Hence γ-tocopherol is:



Synthesis: Both β and γ-tocopherols have been synthesised by methods analogous to those used in the synthesis of the α-isomer. Thus isomeric xylo-hydroquinones have been condensed with phytyl derivatives in presence of $ZnCl_2$. Better yields are claimed when the mono-esters *e. g.* benzoates of the quinols, in place of the free quinols are used in the condensation. The ester group is subsequently removed by hydrolysis.

Vitamins D

Occurrence: Cod-liver oil and other fish-liver oils contain a fat-soluble principle which cures the disease called rickets. The liver oils from the members of the perco-morph (mackerel) family contain the same anti-rachitic factor and are even more potent than those from the halibut or cod. Also anti-rachitic, fat-soluble factors are found to be present in many irradiated oils. Such anti-rachitic factors are called vitamins D. So far the five following vitamins: vitamin D_1, D_2, D_3, D_4 and D_5 have been isolated.

Isolation of the Vitamins D_2, D_3 : The story of the discovery and isolation of the vitamin is a scientific classic. During the period when attempts were made to isolate the active anti-rachitic principle from fish-liver oils, it was discovered by Steenbock, Hess and Resenheim that irradiation of foodstuffs with ultravoilet light produces compounds with pronounced anti-rachitic properties. Irradiation of cholesterol and ergosterol gave very potent anti-rachitic preparations. In 1932 Windaus and co-workers actually isolated in a pure crystalline form, the anti-rachitic principle from the crude product of irradiated ergosterol. It is also formed by the bombardment of ergosterol with cathode rays. They called it *calciferol* or vitamin D_2. It melts at 115°–116°. This product, is not identical with the natural anti-rachitic factor of the liver oils, as shown by the bio-assays and other physiological properties. This discrepancy stimulated the study of the irradiation products obtained from other dehydro-sterols and it is now known that the irradiation of 7-dehydro-cholesterol gives a product identical with one of the natural vitamins D. At this stage, the multiple nature of the natural antirachitic factor was being gradually established by the bio-assay and other evidence. Finally, Brockmann effected the isolation of the anti-rachitic principle, vitamin D_3 from the concentrates of both the tuna liver oil and the halibut liver oil. He used the chromatographic method of analysis and from the concentrated product, obtained the 3–5–dinitro benzoate derivative of the vitamin D_3. In its chemical and physiological properties, this substance was identical with the product of irradiation of 7–dehydro–cholesterol.

Thus it is now established that the fish-liver oils contain the anti-rachitic principle vitamin D_3 while the irradiated ergosterol contains vitamin D_2. Other closely related sterols on irradiation give anti-rachitic factors; these sterols are called pro-vitamins. The different forms of vitamin D, with their corresponding pro-vitamins are :

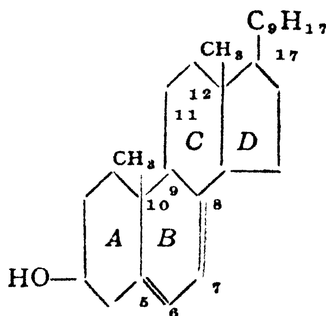
vitamin D_2	ergosterol
vitamin D_3	7–dehydro-cholesterol
vitamin D_4	22–23–dihydro–ergosterol
vitamin D_5	7–dehydro–sitosterol

Vitamin D_1 is found to be a molecular compound of vitamin D_2 and lumisterol. The latter is one of the intermediate products of irradiation of ergosterol.

Vitamin D_2 or calciferol

Isolation : Ergosterol is irradiated till 40-60% of the pro-vitamin has been transformed. The irradiation products is a crude mixture containing vitamin D_2 and other products like tachysterol. The latter is removed by condensing it with citraconic anhydride. The reaction product after treatment with citraconic anhydride, is saponified at room temperature, and extracted with petroleum ether and water; the tachysterol-citraconic anhydride adduct remains in the water phase, while the vitamin passes into the petroleum ether. It is finally crystallised from acetone-methanol mixture. In another method, the vitamin is isolated from the irradiation mixture as the characteristic, sparingly soluble 3–5–dinitrobenzoate. It is purified by fractional crystallisation and the vitamin obtained by saponification of the ester.

Consitution : Vitamin D_2 has the molar composition $C_{28}H_{44}O$ and is thus isomeric with ergosterol, from which it is also derived. The constitution of the vitamin must be closely related to that of ergosterol which is:



I ergosterol

The structure is finally established on the basis of the following evidence.

(*i*) The vitamin forms a mono–ester thus indicating the presence of one OH group.

(*ii*) On catalytic hydrogenation, the vitamin absorbs 4 molecules of H_2 ; this indicates the presence of 4 double bonds.

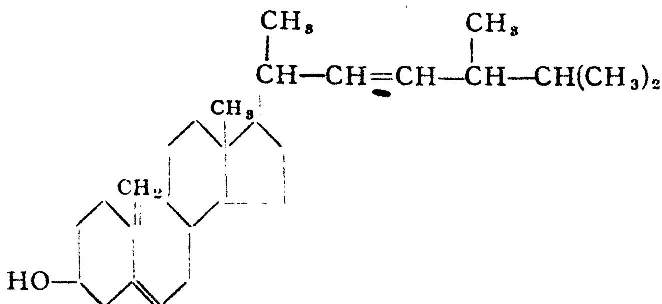
The molecular formula ($C_{28}H_{44}O$) and the presence of 4 double bonds, together suggest that the molecule of the vitamin contains only *three* rings. Now ergosterol contains *four* rings and three double bonds only. These results thus indicate that the conversion of the sterol into the vitamin is accompanied by the opening up of one of the rings (A, B, C or D). That the ring *B* is opened up between C_9 and C_{10} is established by the results of oxidation.

(*iii*) The vitamin on oxidation with cold chromic acid gives (*a*) an aldehyde of the composition $C_{21}H_{34}O$ and (*b*) a ketone $C_{19}H_{32}O$; the aldehyde is an α-β unsaturated carbonyl derivative as indicated by the absorption spectrum of its semi-carbazone. It contains no hydroxyl group, but has a high carbon content (C_{21}) ; it is therefore obvious that it can only come from the cleavage of the double bond in 5-6 position in I resulting in a structure with the ring *B* opened up.

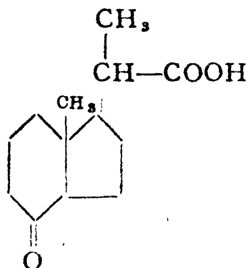
The ketone $C_{19}H_{32}O$ is monounsaturated ; the composition thus indicates the presence of two rings. The number of C atoms present indicates that the two rings contain the original side-chain and the original angular methyl group between the rings C and D. The keto group is therefore attached to what was originally the ring *C*. In ergosterol, ring *C* is linked up with ring *B* through C_8 and C_9. The keto group in the reaction product from the vitamin indicates that in the latter, only one linkage exists between ring *C* and what was ring *B* in the ergosterol molecule, and that the ring *C* is linked up with the rest of the molecule by a double bond.

The above results thus clearly show that in the vitamin, the ring B of the ergosterol molecule does not exists ; the cleavage has occurred between C_9 and C_{10} because, a keto group at C_9 is impossible as a double bond cannot exist between C_9 and C_{10} (C_{10} is quaternary in ergosterol). Hence in the vitamin, a double bond must exist between C_7 and C_8. This establishes the nature of the rings C and D.

(*iv*) The vitamin forms an adduct with maleic anhydride; the latter on hydrolysis, gives a dibasic acid which on dehydrogenation with Pt, forms naphthalene and β-naphthoic acid. The diester of the dibasic acid on dehydrogenation with Se, gives 2-3-dimethyl-naphthalene. These dehydrogenation results indicate that maleic anhydride addition, leads to the formation of a hydronaphthalene derivative. This is possible only, when the two unaccounted for double bonds, which are conjugated with the double bond between C_7 and C_8, are present between C_5 and C_6, and C_{10} and C_{19}. On the basis of the above results, the following structure is assigned to vitamin D_2 or calciferol.



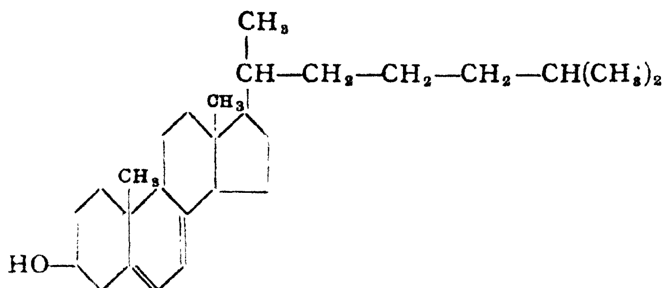
The above structure is in complete agreement with the results of oxidation with ozone. Thus, the vitamin on oxidation with ozone, gives CH_2O, and a keto-acid :



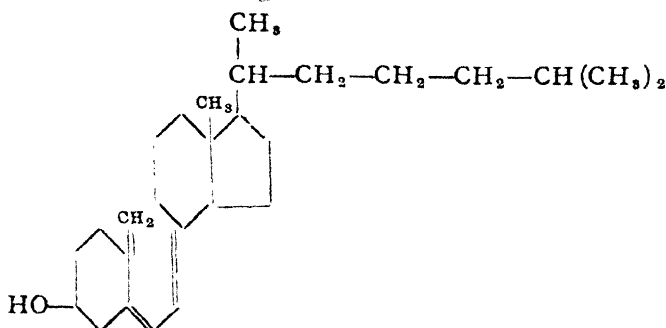
which is obtained by cleavage at the double bonds between C_7 and C_8 and C_{22} and C_{23}.

Vitamin D_3

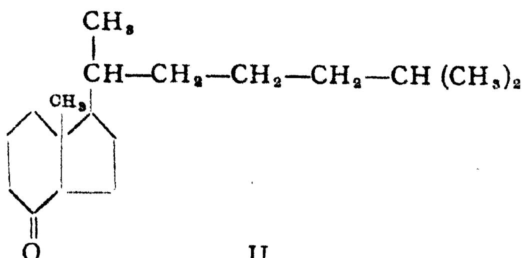
This vitamin is obtained from 7-dehydrocholesterol, in the same way as vitamin D_2 is obtained from ergosterol. Hence, it is argued that it is structurally related to 7-dehydrocholesterol, as vitamin D_2 is to ergosterol. The sterol has the structure :

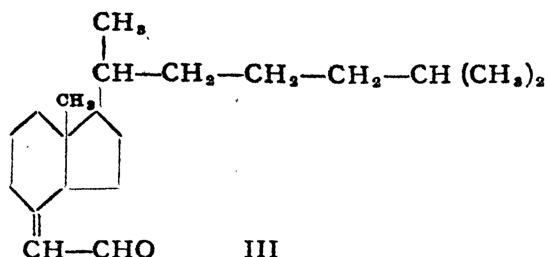


Hence the structure of the viamin D_3 :



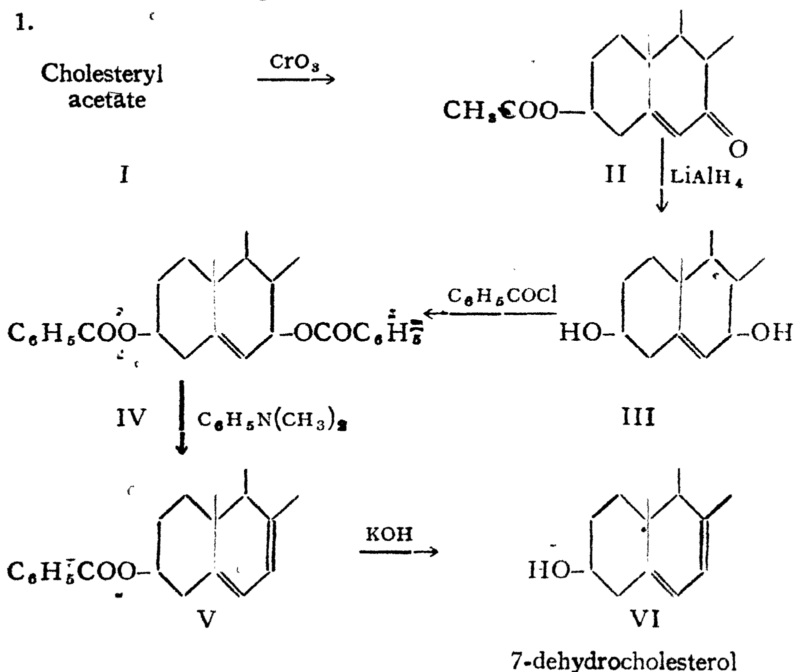
The above structure is confirmed by the results of ozonolysis. A saturated ketone II and an unsaturated aldehyde III are obtained, as indicated by the structure.





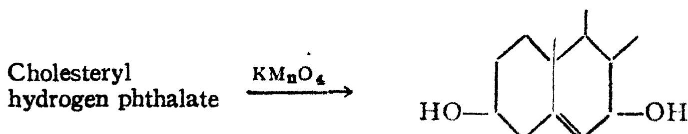
This vitamin is identical with the anti-rachitic principle isolated from the natural fish-liver oils. It forms colourless crystals, m. p. 82·4°. 7-Dehydrocholesterol has been obtained from cholesterol by one of the following methods :

1.



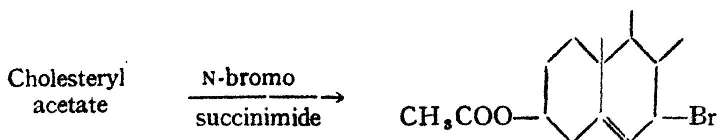
7-dehydrocholesterol

2.



which is then converted into the dibenzoate (IV) which is then converted into 7-dehydrocholesterol as under 1.

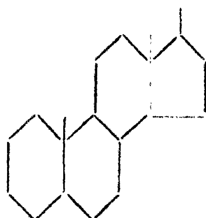
3.



The 7-bromo compound on treatment with $C_6H_5N(CH_3)_2$ gives the 7-dehydro-cholesteryl acetate; on hydrolysis it gives the 7-dehydro-cholesterol.

Vitamin D_4 is obtained from the irradiated 22-dihydro-ergosterol; vitamin D_5 is derived from 7-dehydro-sitosterol.

All these vitamins D_2, D_3, D_4 and D_5 are thus, structurally closely related to cholesterol, the principal sterol. The sterols, the bile acids, the aglucons of the cardiac glycosides, the sapogenins and sex-hormones comprise a family of naturally occurring compounds which are derived from a common system. They are the oxygenated and substituted derivatives of a parent hydrocarbon called "cyclo-pentano-perhydro-phenanthrene."



Vitamins K_1 and K_2

Occurrence: Dam in the course of his experiments on cholesterol metabolism in chicks, noticed a hæmorrhagic condition in the chicks fed on ether extracted diet. Subsequently, he showed that this condition was not due to the deficiency of vitamins already known *e. g.* A, B_1, B_2, C and D_2 but to the lack of a new fat soluble factor which he termed vitamin K (Koagulations-vitamin), *i. e.* blood-coagulating vitamin. At present, two compounds possessing anti-hæmorrhagic activity, have been isolated and are known as

vitamin K_1 and vitamin K_2. The most important sources of these vitamins are:

(*a*) Vitamin K_1: green leaves and vegetables *e. g.* alfa-lfa, chest-nut leaves, cabbage, cauliflower, spinach etc.

(*b*) Vitamin K_2: putrefied fish meal.

Isolation: The source material for vitamin K_1 is the dried alfalfa meal; vitamin K_2 is obtained from the putrefied fish meal. The isolation of the vitamins K_1 and K_2 has been rendered possible, in a high degree of purity, by the application of the chromatographic methods of adsorption. An excellent procedure based on this principle is developed by Binkley and others who use *permutite*, *decalso* and *dărco* as the adsorbents. The vitamins are stable towards these adsorbents and the adsorption is almost quantitative. Both the vitamins have thus been obtained in *crystalline* form. The crystals of vitamin K_1 melt at—20°; those of K_2 melt at about 54°; at ordinary temperature, vitamin K_1 is a yellow, oily liquid.

Constitution of Vitamin K_1: The molecular composition is $C_{31}H_{46}O_2$; it gives the following typical reactions:

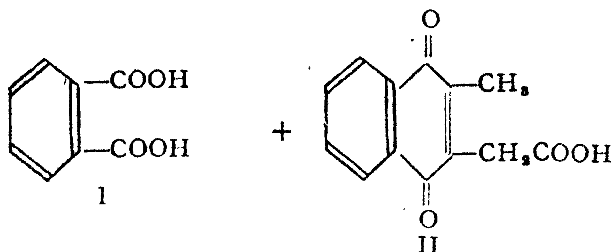
(*a*) It is yellow and is easily reduced with sodium hydrosulphite ($Na_2S_2O_4$), to a colourless dihydroxy derivative; the reduction is also reversible. These results show that it is a 1 : 4 quinone.

(*b*) A study of the ultra-violet absorption spectrum of the vitamin shows that it is related to 2, 3 disubstituted 1·4 naphtho quinones.

(*c*) Its anti-haemorrhagic activity also suggests 1 : 4 naphthoquinone structure, because 1 : 4 naphthoquinones are known to possess anti–hæmorrhagic activity.

The exact structure of the molecule is then evolved from the results of oxidative degradation.

Vitamin K_1, on oxidation with chromic acid yields a mixture of two acids I and II.

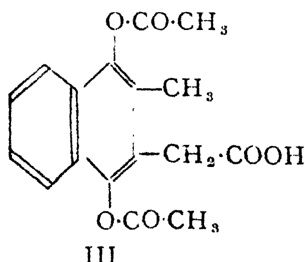


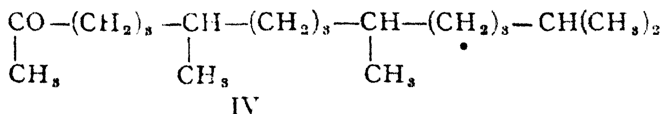
The structure of II as methyl-1·4 naphthoquinone-3 acetic acid has been established by an actual synthesis of the acid and comparing it with the degradation product.

These results thus prove that the vitamin is a 1·4 naphthoquinone derivative carrying a CH_3 group in position 2 and another substituent in position 3; the nature of the substituent in position 3, is revealed by the oxidation studies of diacetyl-dihydro vitamin K_1.

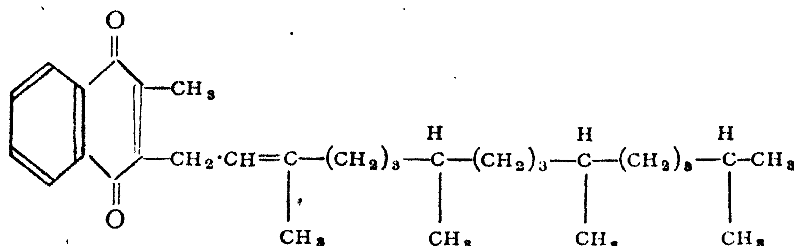
The latter is obtained by the reductive acetylation of the vitamin, with zinc, acetic anhydride and Na-acetate.

Diacetyl-dihydrovitamin K_1 on oxidation with chromic acid, gives an acid III and a ketone IV.





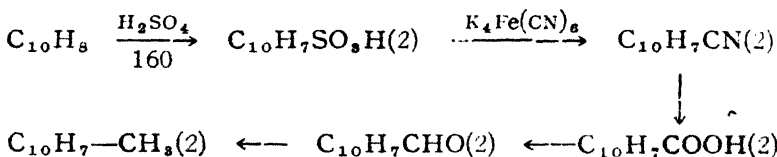
The above ketone IV is found to be identical with the phytyl ketone $C_{18}H_{36}O$, obtained by the ozonolysis of phytol. Hence the side-chain in position 3, is derived from the phytyl radical and must be linked through the C atom which appears as CO in IV, to the C atom appearing as COOH in the acid III as indicated below:



i. e. 2 methyl-3 phytyl-1-4 naphthoquinone.

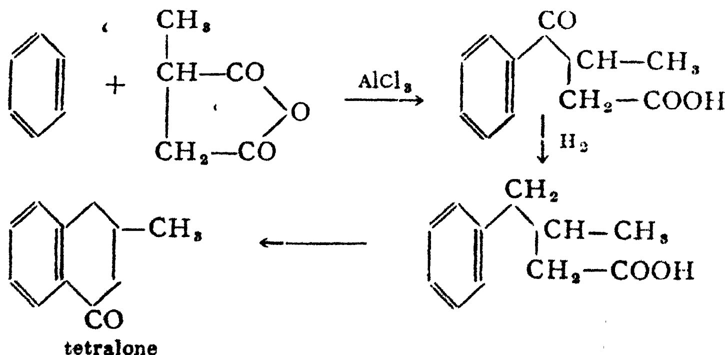
Synthesis : Phytyl bromide is condensed with a benzene suspension of the mono-sodium salt of 2 methyl-1-4 naphthohydroquinone ; the product is subsequently oxidised by atmospheric oxygen to give the vitamin. In another method, 2 methyl-1-4 naphtho-hydroquinone is condensed with phytol in dioxane solution, in presence of oxalic acid. The 2-methyl-1-4 naphtho-hydroquinone required in the above synthesis is obtained by the following methods :

1. The starting point is naphthalene :



The last compound on oxidation with chromic acid gives 2-methyl-1-4 naphthoquinone ; on reduction with $Na_2S_2O_4$, it gives the required 2-methyl-1-4 naphtho-hydroquinone.

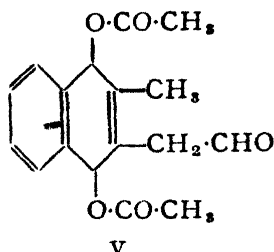
2. The starting point is benzene :



The tetralone on reduction and subsequent dehydrogenation with Se, gives 2-methylnaphthalene, which can be converted into 2-methyl-1-4 naphtho-hydroquinone as described above.

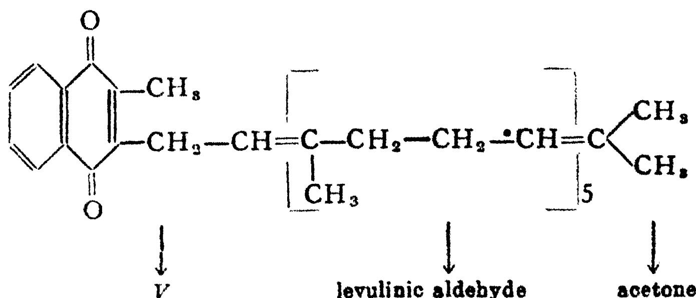
The methyl succinic acid required in this synthesis is obtained by distilling tartaric acid.

STRUCTURE OF VITAMIN K_2 : The structure of this vitamin has been investigated on the same lines as its close analogue. The ozonolysis of the diacetate of dihydro-vitamin K_2 yields levulinic aldehyde, acetone and another compound (V).



V

These results prove that vitamin K_2, like the vitamin K_1 is a 2 methyl-1-4 naphthoquinone derivative. The nature of the substituent in position 3 must be such that levulinic aldehyde (5 molecules) and acetone are produced on ozonolysis. The following formula is in agreement with the above facts :—



Thus vitamin K_2 is 2-methyl-3-difarnesyl-1-4 naphthoquinone. So far its synthesis has not been reported.

SYNTHETIC ANTI-HAEMORRHAGIC PRODUCTS: A large number of synthetic products related to 1·4 naphthoquinone have been obtained and found to be antihæmorrhagic in their action. Thus 2-methyl 1-4 naphthoquinone and 2 methyl-3-alkyl 1-4, naphthoquinones (where the alkyl group is a radical containing more than 8 C atoms) show marked potency. Similarly, compounds which on oxidation, give 2 methyl-1-4 naphthoquinone or a related derivative have been prepared and found to possess great antihæmorrhagic activity. A few such important compounds are (*a*) 1-amino-2 methyl-naphthalene, (*b*) 2 methyl-1-naphthol, (*c*) 4-amino-2-methyl-1-naphthol, (*d*) 2 methyl-1-4, naphtho-hydroquinone; of all the synthetic products, the most commonly used is 2 methyl-1-4 -naphthoquinone; it is called by the name "menadione."

Folic acid or Vitamin Bc

Occurrence : Folic acid or pteroylglutamic acid is present in green leaves, yeast and liver. It is the liver *L. casei* factor. Its physiological function is connected with the formation of red blood corpuscles, especially the bone marrow.

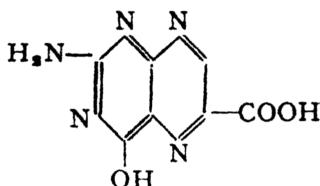
ISOLATION :—The spinach is extracted with water, acidified to pH_3, and the extract stirred with animal charcoal and filtered. The adsorbate is eluted with hot 2·8% ammonia and the eluate readsorbed. The adsorption and elution are repeated a number of times and the vitamin is finally purified by precipitating it as Pb and Ag salts.

CONSTITUTION :—The constitution of the vitamin is established both by analytical and synthetic methods. The analytical method is based on the isolation and identification of the degradation products of hydrolysis.

(*a*) Under ærobic alkaline conditions, the vitamin is split up into two equimolar amounts of (*i*) a highly fluorescent compound *A* and (*ii*) another compound *B*.

The compound *A* has the molar composition $C_7H_5N_5O_3$; it is a dibasic acid and on heating suffers decarboxylation and gives a monobasic acid; on oxidation with chlorine water, followed by hydrolysis with 0·1 N HCl the compound *A* gives guanidine. This fact, in conjunction with the nature of the ultra-violet absorption spectrum and fluorescence, suggested the presence of 2-amino-

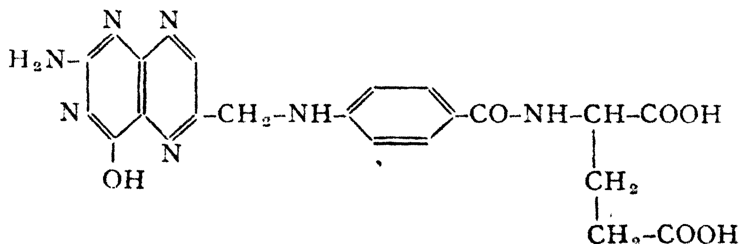
pteridine derivative with a hydroxyl and carboxyl group. Finally, the compound *A* is shown to be 2-amino-4-hydroxyl-pteridine-6-carboxylic acid.



The compound *B*, on acid hydrolysis gives *p*-amino benzoic acid and an α-amino acid: glutamic acid.

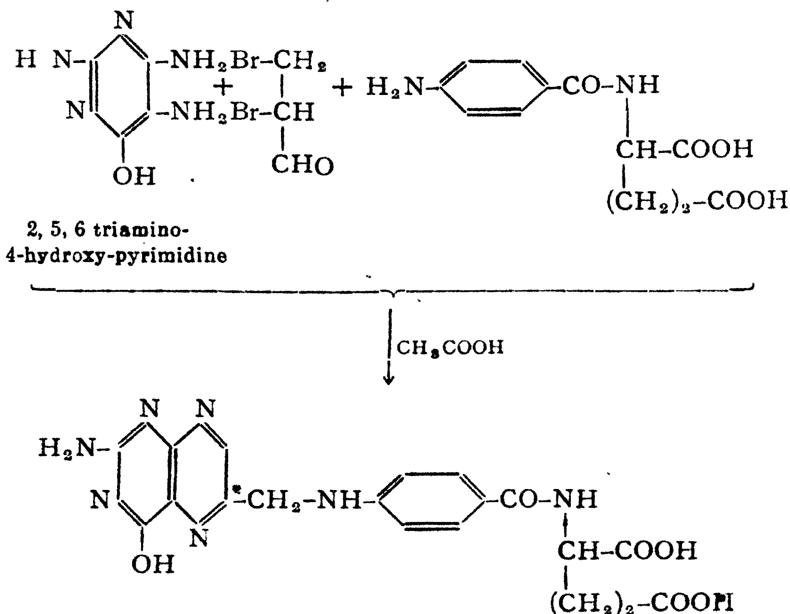
(*b*) The vitamin on treatment with sulphurous acid. gives a pteridine fraction and an aromatic amine derivative. The pteridine fraction on standing in dilute alkali, in absence of air, gives 2-amino-4-hydroxy-pteridine-6-carboxylic acid and 2-amino-4-hydroxy-6-methyl pteridine. The aromatic amine fraction, on alkaline hydrolysis, gives *p*-amino-benzoic acid and glutamic acid.

The above results thus clearly indicate that the vitamin is built up of three moieties ; pteridine, *p*-amino-benzoic acid and glutamic acid. The simultaneous formation of the pteridine moiety and the aromatic amine suggests that the former is linked to the NH_2 of the *p*-amino-benzoic acid, through a CH_2 group in position 6 (the position of the COOH in the pteridine moiety). This is in agreement with the observed necessity of oxygen in the alkaline cleavage of the vitamin. The hydrolysis of N-benzyl-*p*-amino-benzoic acid with alkali, is accelerated by the presence of oxygen. Hence the structure for the vitamin is :



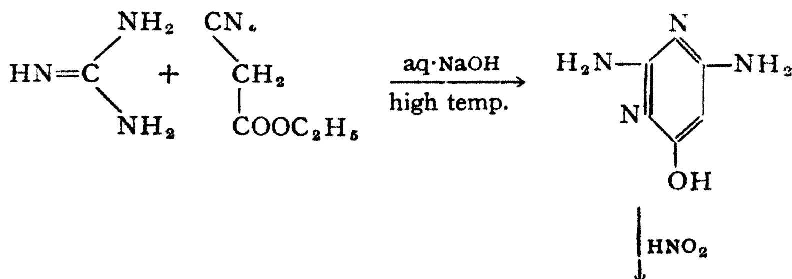
SYNTHESIS :—The molecule of folic acid is built up of three moieties : (*i*) pteridine system, (*ii*) *p*-amino benzoyl-group and

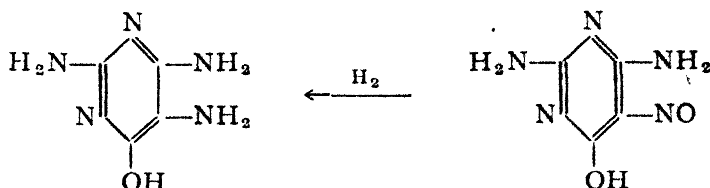
(*iii*) glutamic acid residue. The synthesis of the vitamin by Angier involves the following steps :



In a modified procedure, 2, 3 dibromo-propionaldehyde is first converted into the pteridinium bromide derivative which is then condensed with *p*-aminobenzoyl-glutamic acid, in glycol medium in presence of $NaOC_2H_5$ at 140°, to give the vitamin.

The triamino-hydroxy-pyrimidine derivative is obtained by the following series of reactions :





HORMONES

Introduction. Bayliss and Starling who were the first to investigate the secretion of the pancreatic juice, established that it was the effect of a chemical stimulus and not a nerve stimulus as in the case of saliva secretion. They suggested the term "hormones' for compounds of this class. They are the specific compounds' produced by the ductless glands, such as the thyroid, adrenals, the pancreas, and the pituitary. They find their way into the blood stream and seem to influence and regulate the functions of other organs of the body. Hence the name, hormones which means to excite. They are sometimes referred to as 'chemical messengers" They are of immense physiological importance. They are highly specific in their physiological action, and are required in small quantities; the hormonal deficiency leads to a definite pathological condition, which can be cured by the administration of the specific hormone.

Chemically, they belong to widely different groups of compounds; they may or may not contain nitrogen. Some of them are phenolic and a few others are ketonic in nature. They are susceptible to oxidation and to hydrolytic reactions with acids, alkalies or enzymes.

As a class, they possess the same general characteristics as the vitamins (p.), but differ from them in that they are produced by the animals themselves to meet their requirements. A few of the hormones have been isolated from the corresponding ductless glands, and their structures well established by complete and unambiguous synthesis. The typical glands and their secretion products *i. e.* hormones are given below :

The adrenal glands :

(*i*) the medulla ⟶ adrenaline
(*ii*) the cortex ⟶ cortex hormone.

The thyroid gland ⟶ thyroxine

The pituitary gland ⟶ pituitrin

The pancreas ⟶ insulin.

Lastly there are the sex-hormones, elaborated by the glands of the genital systems and which cause important physiological changes. At present, three types of sex-hormones, are recognised. The types are :—(*i*) estrogenic hormones, estrogens. (*ii*) corpus luteum hormones, gestogens and (*iii*) androgenic hormones (androgens).

Adrenaline

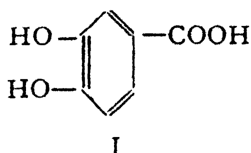
Occurrence. It is the hormone of the adrenal glands; the latter is also called the suprarenal gland, as it is situated above the kidney. The gland consists of two histologically distinct parts : (*i*) the medulla and (*ii*) the cortex. Adrenaline is secreted by the former part. It is also called epinephrine.

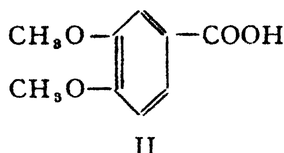
Isolation. It was first isolated from the glands in a crystalline form in 1901, simultaneously by Takamine and by Aldrich. It was the first hormone to be obtained in a pure and crystalline form. The actual procedure of isolation is based on the marked basic character of the compound. The minced glands of oxen are extracted with acidulated water and the extract is heated to coagulate the proteins. The filtrate, after removal of the proteins is concentrated in vacuo. The remaining impurities are then precipitated by the addition of alcohol. The alcoholic filtrate is freed from the solvent by concentration in vacuo, and adrenaline is precipitated by adding ammonia to the concentrated solution. It is finally purified by dissolving it in alcohol containing 15% oxalic acid. During all the above operation, an inert atmosphere is maintained by using a high vacuum or an atmosphere of CO_2 or a layer of petroleum. It is a crystalline compound mp 21%. It is a layer of petroleum

Constitution. The molecular composition of the hormone is $C_9H_{13}O_3N$. Its constitution is based on the following relevant analytical evidence :

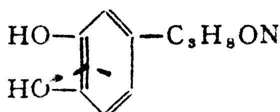
(*a*) It is easily oxidised and gives an intense green colour with $FeCl_3$; this indicates the presence of a catechol unit.

(*b*) On fusion with potash, adrenaline gives proto-catechuic acid (I), while methylated adrenaline gives veratric acid (II) and $N(CH_3)_3$





Thus the presence of a catechol unit and the exact position of the side-chain in the molecule are clearly indicated. Hence adrenaline is:

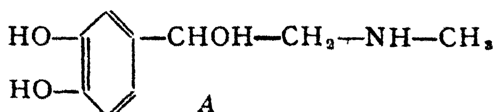


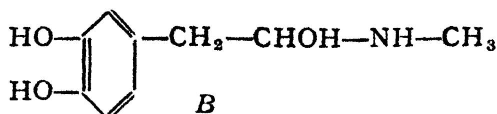
The exact nature of the side-chain is revealed by the following evidence:

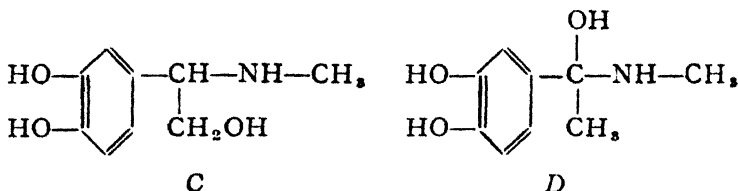
(*i*) On boiling with HCl for a long time, adrenaline gives methylamine; also the oxidation of the methylated adrenaline with $KMnO_4$ gives methylamine along with veratric acid. This indicates the presence of $NH\text{-}CH_3$ group in the side-chain. The formation of $N(CH_3)_3$ from methylated adrenaline on fusion with alkali also indicates that the N atom must be at the end of the side chain.

(*ii*) The compound does not react with any carbonyl reagent; hence the oxygen atom in the side-chain is probably present as a hydroxyl group (the other two oxygen atoms are present as phenolic hydroxyl groups).

All the above evidence leads to the formulation of the following four formulæ for the adrenaline molecule:



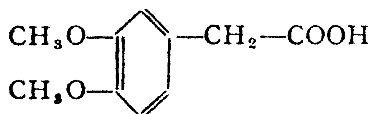




The final choice between the four, is made by the following considerations.

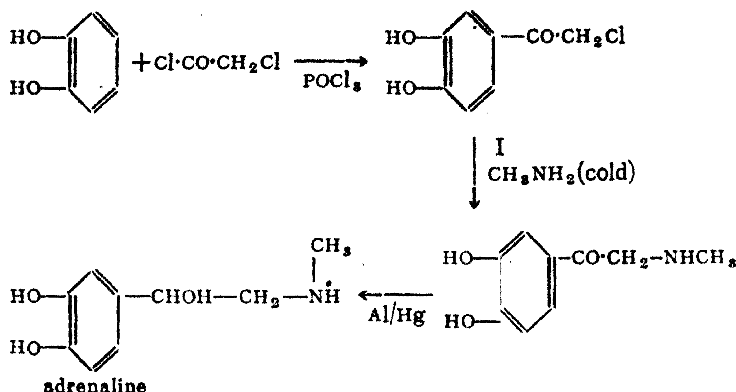
(*i*) When adrenaline is fused with potash, small quantities of pyrrole and skatole derivatives are formed. Formulas C and D cannot account for this observation.

(*ii*) If formula B were true, the oxidation of methylated adrenaline, would give homoveratric acidic a phenyl acetic acid derivative.



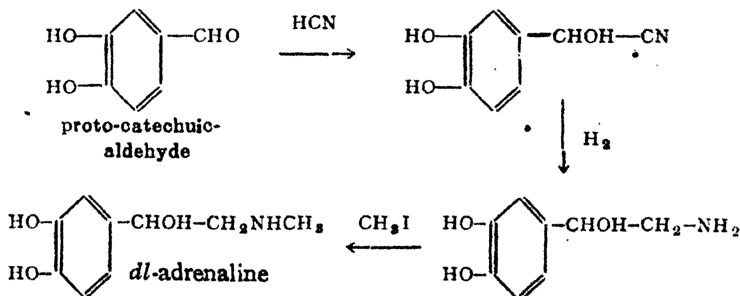
Actually, veratric acid is formed. Hence the choice is for the formula A. This was first confirmed by a synthesis of adrenaline by Stolz. Later on, Friedmann furnished an analytical proof for the exact location of the hydroxyl group in the side-chain. He prepared a tribenzene-sulphonyl derivative of adrenaline by treating it with $C_6H_5SO_2Cl$, which on oxidation gave a ketone; this excludes formulæ B, C and D.

Synthesis (Stolz). Catechol is condensed with chloroacetyl chloride in presence of $POCl_3$ to give chloroacetyl catechol (I); the latter is treated with a large excess of concentrated solution of methylamine to form the ketone, adrenalone (II). The ketone on reduction with Al-amalgam gives racemic adrenaline. The yield however is poor.



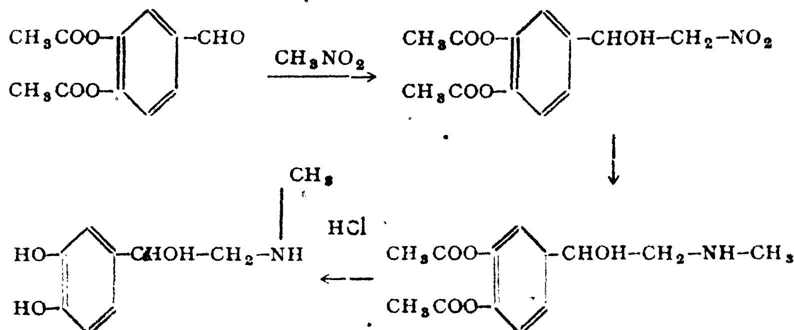
Subsequently, some improvements have been introduced to increase the yields. Equimolar quantities of catechol and chloroacetyl chloride are heated together in boiling benzene, to form the ester, chloroacetate of catechol; the latter on heating with 5 to 10% of its weight of $POCl_3$ gives chloroaceto-catechol (I) which is then converted into adrenaline as above. This modification greatly reduces the tar formation observed in the earlier synthesis, in the direct condensation of the three reactants. Another modification consists in the use of H_2 in presence of Pd to convert the ketone into the hormone.

Another synthesis of adrenaline starts from proto-catechuic-aldehyde. The different steps in the synthesis are :—



The following synthesis of adrenaline is also interesting. The diacetyl derivative of proto-catechuic aldehyde is condensed with

CH_3NO_2 in weakly alkaline solution, when a phenyl-nitroethane derivative is formed. The latter is treated with CH_2O and reduced with nascent hydrogen (Zn and CH_3COOH) to form a diacetyl derivative of adrenaline, which on hydrolysis gives adrenaline :—



The synthetic product which is racemic, is then resolved by means of *d*-tartaric acid. When *dl*-adrenaline-*d*-tartrate is heated with CH_3OH, *d*-adrenaline-*d*-tartrate dissolves and the *l*-adrenaline-*d*-tartrate remains behind. The latter is further purified by crystallisation from 95% methanol, and decomposed with NH_3 to give to the free l-adrenaline. *d*-Adrenaline is racemised by heating it with HCl and again resolved as above to give the more active *l*-base.

Despite all the improvements in the synthetic production of adrenaline, the natural product from the American highly organised packing houses, continues to compete with it successfully in the market.

Thyroxine

Occurrence. Thyroxine occurs in the peptide: thyreo—globulin from which it can be obtained by hydrolysis. It is the iodine bearing hormone of the thyroid gland which controls the metabolism of animal organisms. The iodine content of animals is mostly present as thyroxine. Deficiency of iodine causes the disease called goiter. It was first isolated by Kendall, and later on by Harington.

Isolation. The dried gland is hydrolysed in two stages by first using a hot 10% solution of $Ba(OH)_2$ and then a 40% solution of $Ba(OH)_2$. After the first hydrolysis, some thyroxine separates out as the barium salt, and the whole amount is thrown down as the barium salt after the second. The barium salt is then decomposed by suspending it in hot 1% NaOH solution, and adding a slight excess of Na_2SO_4 solution. Barium sulphate is then removed by filtration, and the filtrate containing the sodium salt of thyroxine is acidified while hot, to give thyroxine. It is finally purified by converting it into a sodium salt by dissolving it in alcoholic alkali and precipitating the hormone with acetic acid; the hormone obtained by the above method is racemic and melts at 231°. A small amount of the *l*-isomer was obtained by Harington, by decomposing the racemic form with trypsin.

Constitution. The molecular composition of thyroxine is $C_{15}H_{11}O_4I_4N$. It was Harington who determined its molecular composition and established its structure. In collaboration with Barger, he finally achieved a synthesis of the hormone. The analytical evidence on which the constitution is based is as follows:

(*i*) Thyroxine is a monobasic acid and hence, must contain a —*COOH* group. It can be readily acetylated, which indicates the presence of *OH* or NH_2 groups or both. Thyroxine is a product of hydrolysis of a natural protein; therefore, it should be an α-amino acid. The presence of NH_2 group is further confirmed by the action of nitrous acid; with the latter, the whole of the nitrogen present is evolved as nitrogen gas.

(*ii*) The nature of the carbon framework is revealed by the degradation products of thyronine $C_{15}H_{15}O_4N$, also called desiodo thyroxine; it is obtained from thyroxine by reduction with H_2 in alkaline medium in presence of colloidal Pd. The study of sucha derivative is necessitated by the fact that as iodine forms 65% of the molecule, the analytical data are difficult to interpret, the degradation products which would contain iodine, would also increase the difficulty of interpretation, as the necessary corresponding reference compounds are not available.

CONSTITUTION OF THYRONINE : it is based on the following analytical evidence :

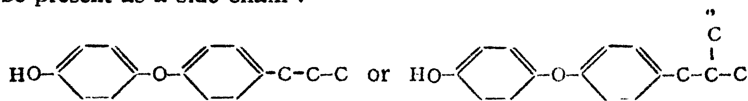
(1) Thyronine gives a brick red colouration with Millon's reagent (a solution of Hg in HNO_3 acid). This indicates the presence of a hydroxy-phenyl (HO—⬡—) group.

(2) Thyronine gives the ninhydrin test which is characteristic of ∝-amino-acids. Hence thyronine is an ∝-amino acid.

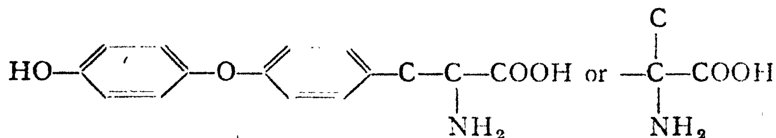
(3) On fusion with potash at about 300°C, thyronine gives the following decomposition products.

(*i*) quinol,
(*ii*) *p*-hydroxy-benzoic acid,
(*iii*) a diphenyl ether, $HO—C_6H_4—O—C_6H_4—CH_3$,
(*iv*) $(COOH)_2$ and NH_3.

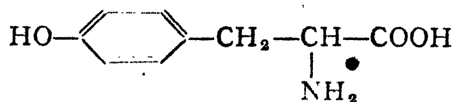
These results indicate that the molecule of thyronine contains two benzene nuclei linked as an ether—$C_6H_4—O—C_6H_4$ and one of the nuclei carries a hydroxyl group in para position to the ether linking. The remaining three carbon atoms ($C_{15}—C_{12}=C_3$) must be present as a side-chain :—



but thyroxine and therefore, thyronine is an ∝-amino acid; hence we have :—



The exact nature of the side-chain is indicated by the reaction with concentrated hydriodic acid. (*a*) On boiling with this reagent, thyronine gives tyrosine :—



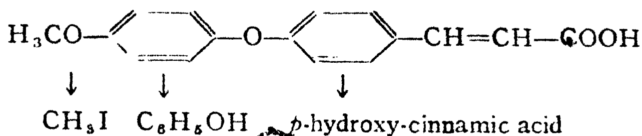
tyrosine

(*b*) On exhaustive methylation, thyronine gives an unsaturated acid $C_{16}H_{14}O_4$ and $N(CH_3)_3$.

The unsaturated acid gives a dibromo addition compound with Br_2, thus it contains only one double bond which indicates that nitrogen formed the part of an open-chain system. (See exhaustive methylation.) On treatment with *HI*, the unsaturated acid gives:—

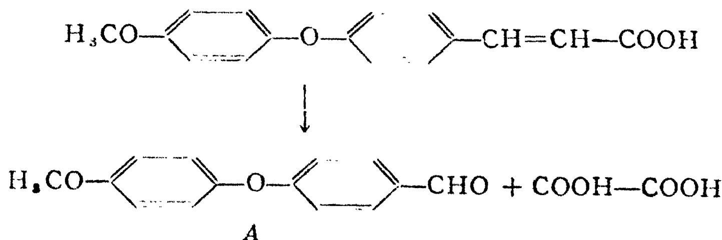
(*a*) C_6H_5OH, (*b*) CH_3I, and (*c*) *p*-hydroxy-cinnamic acid.

The unsaturated acid can, therefore, be best represented by :

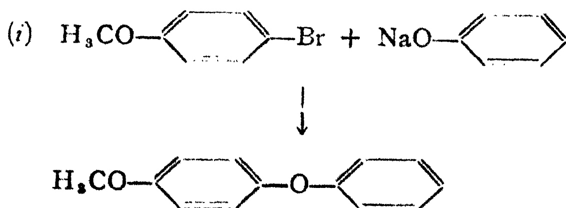


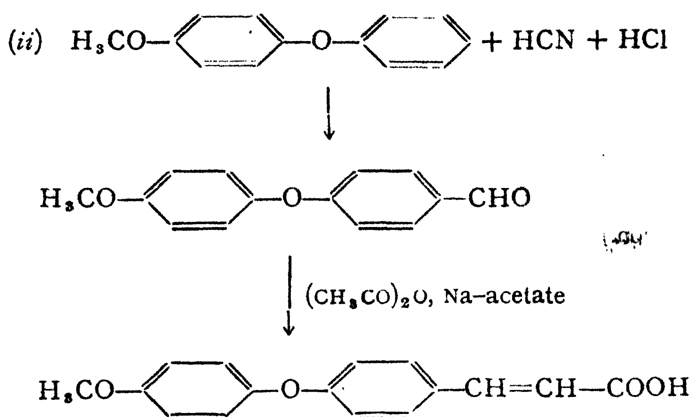
The above formula is further corroborated by oxidation results and by synthesis.

The oxidation of the unsaturated acid with potassium permanganate, would give an aldehyde (*A*) and oxalic acid :—



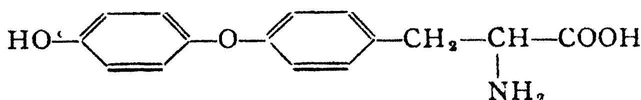
Finally, the acid itself is synthesised by the following steps which establishes its constitution.





All the above evidence thus conclusively establishes the nature of the carbon skeleton (including the side-chain) in the thyronine molecule.

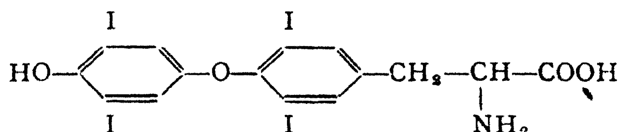
The position of the N atom ;—Thyronine contains a free amino group and as thyronine is simply related to thyroxine which is an α-amino acid, the NH_2 group must be on the alpha-carbon atom. Hence, the formula for thyronine may be written as :—



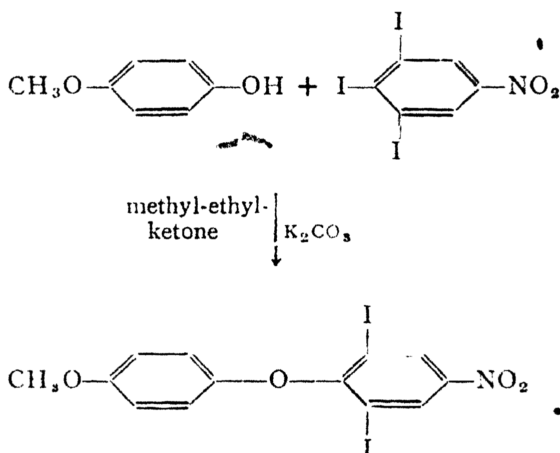
But thyroxine is the tetra-iodo derivative of thyronine. The positions of the iodine atoms are indicated by results of fusion with potash.

Thyroxine on fusion with alkali, gives two products which give pyrogallol reactions. The iodine atoms are replaced by hydroxyl groups and the formation of pyrogallol therefore indicates that two iodine atoms are in ortho positions to the original hydroxyl group in the nucleus, and the other two, in the other nucleus; otherwise, a penta hydroxy benzene derivative would have been formed. Further, thyroxine gives with nitrous acid a yellow colouration, which deepens on boiling and is changed to red when cooled and rendered alkaline

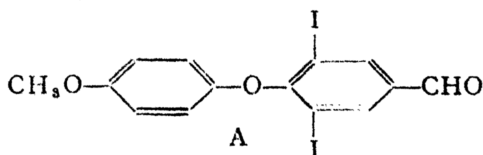
with ammonia. This reaction is specific for phenols iodinated in both the ortho positions. Hence thyroxine has been assigned the formula :



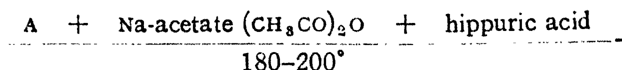
Synthesis. Harington and Barger confirmed the structure by the following synthesis :

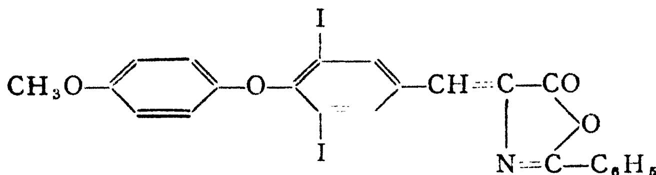


The nitro-compound on reduction with Sn and HCl, gives the aminoderivative, which on diazotisation and subsequent treatment with KCN and Cu_2CN_2, yields the nitrile; the latter on treatment with anhydrous $SnCl_2$ in ether and dry HCl gas, gives the aldehyde (A) :

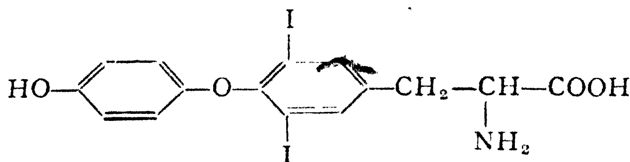


The aldehyde is then heated with hippuric acid, acetic anhydride and Na-acetate. This is the Erlenmeyer's azlactone method.

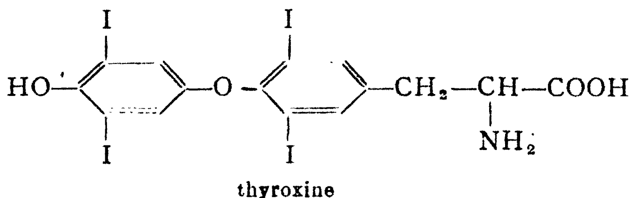




Heating the above, with HI and P, involves demethylation, reduction and hydrolysis and 3, 5 di-iodo-thyronine is formed :



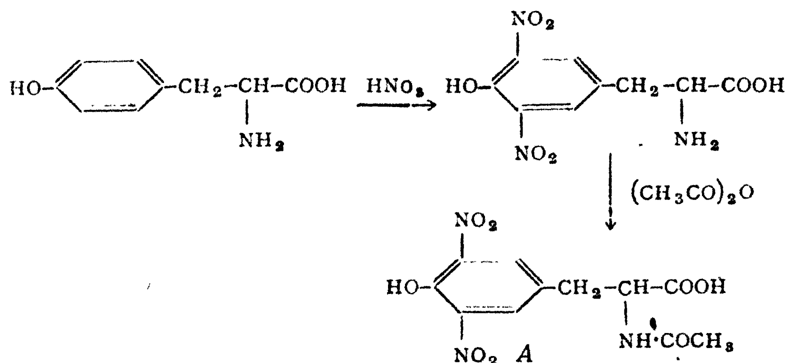
Iodination with I_2 in KI in ammoniacal solution gives the hormone (the racemic compound).



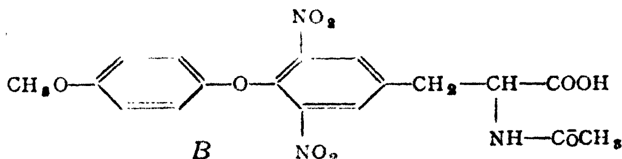
thyroxine

Thy synthetic acid is a racemic compound. On resolution, the *l*-acid obtained is identical with the natural thyroxine in all its properties. The resolution is effected by means of *l* α phenyl-ethyl-amine. The thyroxine obtained by the enzymatic hydrolysis of thyreoglobulin is *l*-rotatory ; it is more active than the synthetic racemic-compound. The racemic thyroxine, on account of its insolubility is very difficult to resolve. Harington overcame this difficulty by resolving the formyl derivative of 3, 5 di-iodo-thyronine and subsequently iodinating the *l* and *d* forms to the corresponding *d* and *l* thyroxine.

Recently the Glaxo Laboratories have announced a new synthesis, which involves the following essential steps :



The *p*–toluene sulphonate of *A* is quaternised with pyridine and subsequently condensed with *p*–methoxy–phenol to give *B*. *B* is



catalytically reduced to the corresponding diamino derivative; the latter is then tetrazotised with nitrous acid in presence of con. H_2SO_4 and acetic acid, and treated with KI to give the di-iodo-derivative, which on heating with HI and acetic acid gives 3, 5 di-iodo-thyronine, which is iodinated to thyroxine with iodine in NaI in presence of ethyl amine. In a recent synthesis, 3, 5 diiodotyrosine in a solution of NaOH at pH 9.5, is heated with stirring at 60° for 18–20 hours, in the presence of MnO_2. There is oxidative coupling of two molecules of 3,5 diiodotyrosine with the elimination of the side chain in one and the formation of thyroxine.

Sex-Hormones

The glands of the male and female genital systems produce compounds which cause the appearance of specific male and female characteristics. They are usually found in the urine which, therefore, is the important source for these glandular secretion products. At

present three different types of sex-hormones are recognised. Thus we have :—(*a*) the œstrogenic or follicular hormones or estrogens (*b*) corpus luteum hormones or gestogens and (*c*) the androgenic or the testicular hormones or androgens.

Oestrogenic Hormones. The most valuable source of these hormones is the urine of both male and female. In the case of the latter, during pregnancy, the content of the hormones is very high. The richest sources for these hormones are, however, the urine of stallion and other males of the Equidæ. Small quantities are present in gonads, ovaries, follicles, and the placenta. Some of these hormones are to be found in the vegetable kingdom also. Thus, œstrone has been discovered in the extract of the palm kernel, and estriol in the pussy willows.

Isolation : The hormonal content of any source is so small that a concentration of about a million-fold of the source material is required. The hormones are usually present in the urine in combination with glucuronic acid from which they are liberated by boiling with concentrated hydrochloric acid. The free hormone is then extracted with a suitable solvent and further purified by fractional solution in different solvents. Girard and Sandulesco have developed the use of a new reagent, trimethyl-amino aceto-hydrazide hydrochloride. It reacts with the ketonic œstrogenic hormones to form derivatives which are soluble in water and therefore, can be readily separated from the accompanying fats and other complex substances. Other methods based either on the formation of additional compounds with quinoline or on adsorption by a suitable adsorbent have also been developed.

So far, as many as seven œstrogenic hormones have been isolated. They are closely related to one another and to cholesterol. They are estrone, estradiols (α and β), œstriol, equilenin, equilin and dihydroequilenin. They are all crystalline compounds and are phenolic in nature. They however, give no colouration with ferric chloride though with sulphonic acids and some other reagents they give characteristic colour reactions.

Constitutions of estrone, estriol, and estradiols (α and β). These three estrogens (which produce estrus i. e. heat) are closely related to one another. Estrone is a phenolic ketone with the composition $C_{18}H_{22}O_2$; estriol has the composition $C_{18}H_{24}O_3$ and

possesses three hydroxy groups, one of which is phenolic and the other two are alcoholic. Further on heating with $KHSO_4$, it is converted into estrone. The estradiols (α and β) are obtained from estrone by reduction in alkaline medium. They have thus no ketonic properties but possess two hydroxy groups, one of which is phenolic and the other alcoholic (formed by the reduction of the carbonyl group).

The typical evidence both physical and degradative, which has led to the establishment of the structural formula of these compounds is as follows :

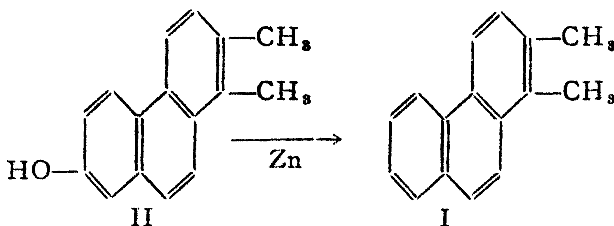
(*i*) Estrone forms an oxime, and an acetate· it is soluble in dilute alkali ; these results indicate that it is a phenolic ketone. The X-ray analysis shows that it is formed of a long molecule with the hydroxyl group at one end and the CO group at the other.

(*ii*) On catalytic hydrogenation, it shows the presence of three double bonds only ; hence it must contain a four-ring system. That the latter is the cyclo-pentano-phenanthrene system is indicated as follows :

(*iii*) A companion compound of estrone, named pregnanediol, isolated by Marrian, has the molar composition $C_{21}H_{36}O_2$; it is a saturated di-secondary alcohol, and contains four-rings. This compound is converted through a series of reactions into a hydrocarbon, pregnane $C_{21}H_{36}$. Butenandt showed that 17-ethyl etiocholane, obtained from bis-norcholanic ester is identical with pregnane. Thus the presence of the cyclopentano-phenanthrene system in pregnane and hence in estrone, the companion compound is indicated.

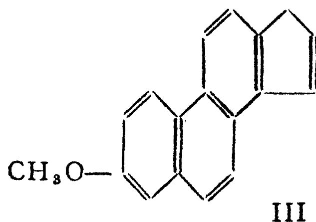
(*iv*) Estriol on fusion with KOH, gives a phenol-di-carboxylic acid containing the same number of C atoms as estriols ; on heating with acetic anhydride, the dibasic acid yields an anhydride and not a cyclic ketone and hence according to Blanc's rule the ring opened up in the fusion is five membered. Further the dibasic acid on distillation with zinc dust, gives 1, 2 dimethyl-phenanthrene (I), while selenium dehydrogenation of the dibasic acid gives a dimethyl-

phenanthrol (II) which can be converted into 1, 2 dimethyl-phenanthrene by zinc-dust distillation :



These reactions thus clearly establish the presence of a phenanthrene skeleton in the degradation product of the hormone. Hence the hormone must have the phenanthrene skeleton with a five membered ring fused on to it, at least, at one of the positions indicated by the two methyl groups in I and II. Further, the hormone is a phenolic ketone; the positions of the hydroxyl and the carbonyl groups are established by the following evidence :

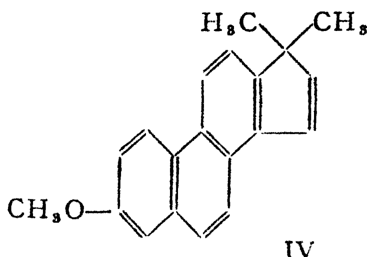
POSITION OF OH. The hormone is first methylated to give a monomethyl ether, which is then reduced by the Clemmensen's method to the des-oxy-estrone methyl ether; on distillation with Se, it gives 7-methoxy-1-2-cyclo pentenophenanthrene (III) whose structure is established by an independent synthesis.



The above results prove conclusively that (*i*) the hormone contains the same ring system as the sterols and (*ii*) that the hydroxyl group is located in position 3.

POSITION OF CO. The methylation product of estrone is treated with CH_3MgI, when a tertiary alcohol is formed. It is successively dehydrated, reduced and dehydrogenated with Se, to form

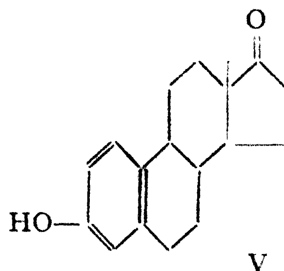
7-methoxy-3'-3' dimethyl-1-2 cyclo-penteno-phenanthrene (IV); its structure was independently established by a synthesis.



This establishes that the carbonyl group—the point of attack by the Grignard reagent is situated in position 17.

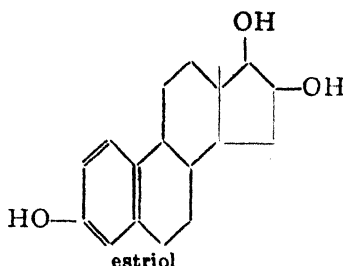
The presence of an angular methyl group at C_{13} is then inferred from the presence of two methyl groups (3'—3') in IV; one is introduced by the agency of the Grignard reagent while the second is due to a rearrangement during the dehydration of the tertiary alcohol formed by the action of CH_3MgI on the ketonic group. Such an interpretation is in agreement with the observation that methyl migration occurs in the dehydration of the dihydroderivative of estrone methyl ether.

Hence, estrone is assigned the structure (V).



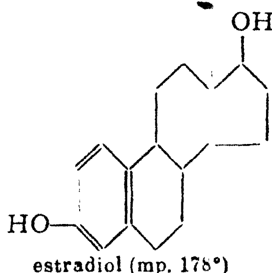
The structure of estriol is then deduced as follows: estriol is a trihydroxy compound and one of the hydroxyl groups is phenolic; on dehydration with $KHSO_4$, it gives estrone, the corresponding phenolic ketone, with the elimination of one molecule of H_2O. Now an α-glycol system is known to yield a ketone on similar dehydration.

Hence the other two hydroxyl groups in estriol must be present as an α-glycol system.



estriol

Estradiol. It is formed from estrone by reduction. Estradiol 17-β, is the exclusive product of reduction with Na and alcohol or of hydrogenation in neutral or alkaline medium. Reduction with Raney Ni, in aqueous KOH, gives a mixture of the α and β estradiols. They are separated by the addition of 1% alcoholic solution of digitonin. The 17-β isomer is quantitatively precipitated as the digitonide. The structure of estradiol is :



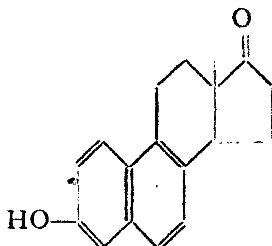
estradiol (mp. 178°)

N.B.—The two estradiols are cis-trans isomers; at first the lower melting isomer was called the α-isomer, in which the C_{17}—OH is trans to the C_{18} methyl group and the other was designated β-isomer, in which the C_{17}—OH is cis to the C_{18} methyl group. But Fieser has recently proposed that the lower melting compound be called 17(β).

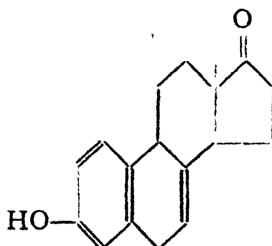
Of these, estradiol 17(β) is physiologically more active and hence, finds use in clinical medicine to relieve the discomfort at menopause. Usually the benzoyl ester (the C_3 hydroxyl is benzoylated), on account of its prolonged effect, is used in preference to the free non-benzoylated hormone.

Equilenin group of estrogens : Equilenin, equilin and 17-dihydro-equilenin are highly unsaturated estrogenic hormones, isolated by Girard, from the urine of pregnant mares. They are found to occur along with estrone to which they are closely related

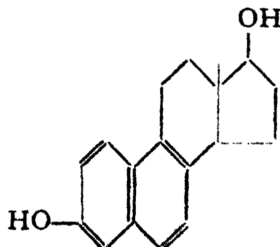
in structure. Equilenin is the most abundant of them all. It has the molar composition $C_{18}H_{18}O_2$; it is a hydroxy ketone and contains two double bonds more than estrone. On methylation and subsequent distillation with Se, both equilenin and equilin ($C_{18}H_{20}O_2$) give 7-methoxy-3′-3′ dimethyl 1·2—cyclopentano phenanthrene, the same compound as is given by estrone under the same conditions. Hence it follows that equilenin and equilin must contain the same steroidal system as in estrone. Further equilenin forms a picrate and hence probably contains a naphthalene system: it has been therefore assigned the structure:



Equilin which contains one double bond more than estrone and is otherwise similar in properties is assigned the structure:



17-Dihydro-equilenine is related to equilenin, in the same way as estradiol is related to estrone. Hence it is assigned the structure:

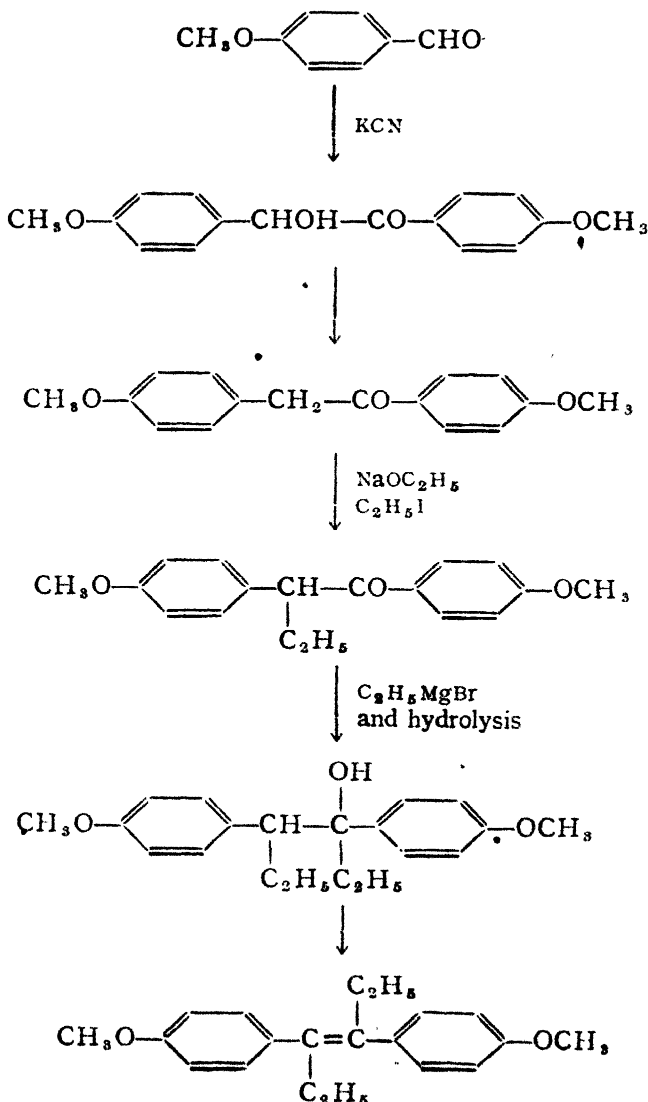


Synthesis of estrone and other estrogens: The total synthesis of estrone, and of estradiol and estriol has been a very difficult problem which still challenges the ingenuity of the organic chemists. The difficulty has been aggravated by the fact that estrone is one of the sixteen possible stereoisomers. Inhoffen has partially succeeded in converting cholesterol, a relatively cheaper and more abundant material, into estrone via androsterone (q. v.). However, the process involves a large number of steps, including the oxidation of a side-chain, a reaction which leads to poor yields, and hence cannot be made the basis for a preparative method for estrone. On the other hand, estrone from urine is very expensive. But in view of the great importance of estrogens in modern therapy, a large amount of research work has been done to obtain nonsteroid synthetic substitutes for estrone and other estrogenic hormones.

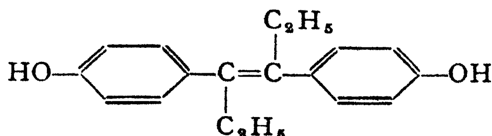
Cook and Dodds investigated a large number of synthetic compounds having some structural similarity to the natural hormone. They found that the estrogenic activity is unspecific and is exhibited by compounds wholly unrelated to estrone. Thus 1-keto-tetrahydro-phenanthrene, 3, 4-benz-pyrene and the diols obtained by the action of Grignard reagents on 1, 2, 5, 6, dibenz-anthraquinone, showed weak estrogenic activity. Later on, Dodds and Lawson reported that a number of diphenyl methane derivatives were estrogenic. But the greatest discovery was made, when Dodds and Lawson found that the demethylation of anethole with KOH and alcohol in a sealed tube, gave a product which was as strongly estrogenic as estrone. Attempts to confirm the above results, however, led to the fact that pure anol (demethylated anethole) possesses very little activity. It followed therefore that the crude product of demethylation obtained by Dodds, contained a highly potent estrogen as an impurity. That the latter may be a product of dimerisation was indicated by the work of Serini and Steinruck, who found that anethole is converted by the action of Grignard reagent into products, which involve demethylation, dimerisation etc. and which exhibit strong estrogenic activity. Dodds, Lawson and others concluded that the active by-product was *stilbestrol*. They synthesised it and actually found it to be very active. The active product in the crude anol was subsequently isolated and found to be hexestrol—the dihydro derivative of stilbestrol. It is more active than the latter. Both the compounds are more active than estrone and approach estradiol

in activity. They further have the additional great advantage that they are effective by oral administration.

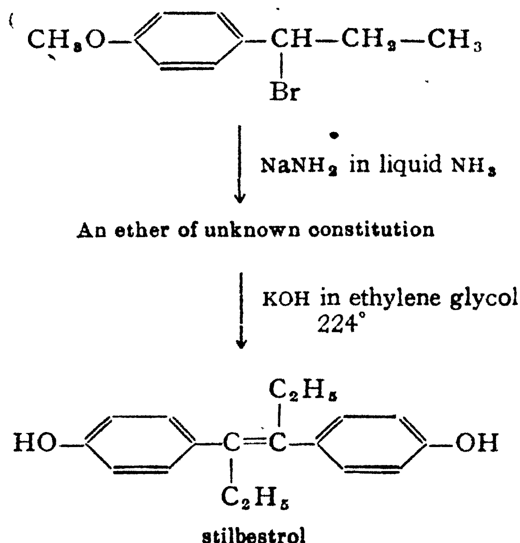
Synthesis of stilbestrol: The original synthesis by Dodds and others, involves the following essential steps :—



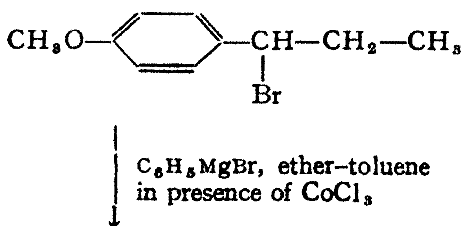
An isomeric *cis* derivative is formed, which in presence of sunlight, is changed into the above *trans* form. The latter on demethylation with alcoholic KOH at 205° gives stilbestrol :—

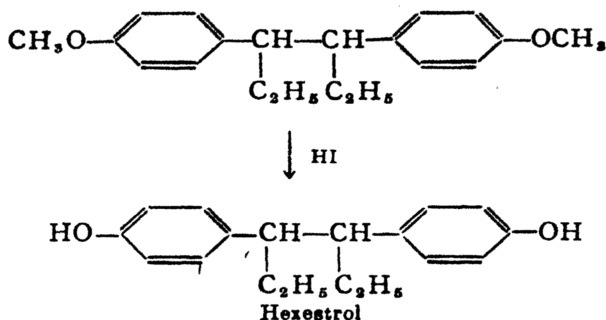


The following synthesis due to Kharasch is superior to many others. Its starts from anethole hydrobromide.



Synthesis of hexestrol: The method of Kharasch involves the following steps :—





Hexestrol

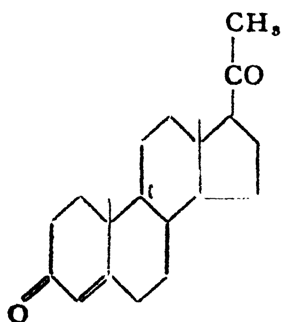
Corpus Luteum Hormone

Occurrence : Progesterone is the hormone secreted by the corpus luteum of the ovarion tissue. Its main function is to prepare for and maintain pregnancy. It is also detected is small quantities, in the placenta and in pregnancy urine; the latter two do not appear to be practicable sources for the hormone.

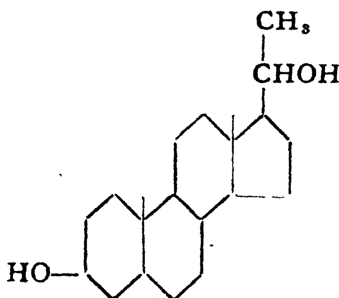
Isolation : It is isolated from the corpora lutea of pregnant sows. The pigs' ovaries are minced and extracted with methanol. The extract is diluted with water to precipitate the fatty matter which is removed by filtration. The filtrate is then extracted with petroleum ether which contains the hormone. The residue left after the removal of the solvent is taken up with 70% ethanol. The alcoholic extract is again diluted with water and extracted with petroleum ether; the progesterone is extracted by the petroleum ether, while some estrone which always accompanies it, is left in the aqueous-alcoholic phase. Progesterone is then obtained in a crystalline form by chilling the petroleum ether extract to—20°C.

Constitution : Progesterone exists in two polymorphic forms : ∝ form (prisms) melting at 128° and the β form (needles) melting at 121°. The molecular-composition is $C_{21}H_{30}O_2$. This suggests a chemical relationship to pregnanediol ($C_{21}H_{36}O_2$) which is known to be 3–20–dihydroxy-pregnane. The above relationship is further elucidated by the following evidence. (i) Progesterone forms a dioxime (ii) Progesterone on catalytic reduction takes up three molecules of H_2 to form $C_{21}H_{36}O_2$. Hence must he a

mono-unsaturated diketone related to pregnanediol. The absorption spectrum of the hormone in the ultra-violet indicates the presence of an α-β unsaturated ketone grouping; the X-ray studies also point to the presence of a sterol-like skeleton in the hormone. On the basis of the above considerations, Slotta proposed the following formula for the hormone, which is related to pregnanediol.



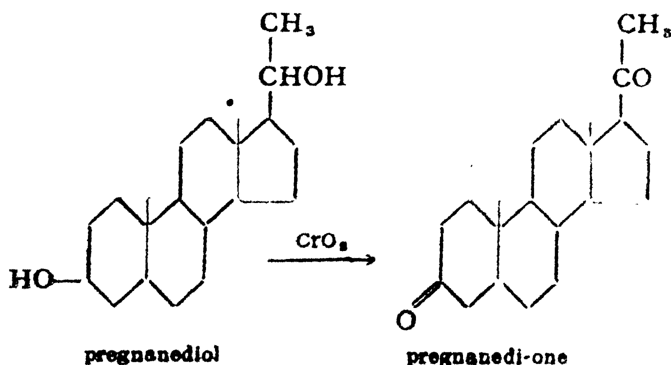
progesterone



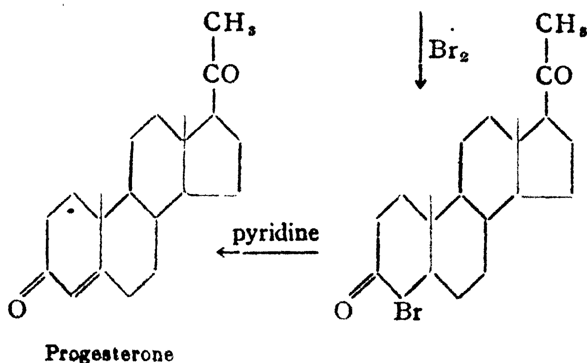
pregnane-diol

Pregnanediol $C_{21}H_{36}O_2$ is a disecondary alcohol and is a saturated compound. On oxidation, it is converted into a diketone which is reduced with Zn/Hg and HCl to pregnane which is 17-ethyl. One of the OH groups is in the side chain (CH_3–CHOH), as it gives the haloform reaction; the other is in 3 position.

The constitution is confirmed by Butenandt, by converting pregnanediol into progesterone. The steps involved in the conversion are :



pregnanediol pregnanedi-one



Progesterone

In another synthesis, stigmasterol is used as the starting material. The essential steps are indicated below :

CH_3—CH—CH=CH

C_2H_5—CH—CH$(CH_3)_2$

HO—

1. acetylation
2. bromination
3. oxidation (O_3)
4. debromination (Zn + acetic acid)

CH_3

CH—COOH

HO—

ester + C_6H_5MgBr and dehydration

CH_3

C=C$(C_6H_5)_2$

HO—

(i) ozonisation of the acetate dibromide

(iii) deacetylation

CH_3

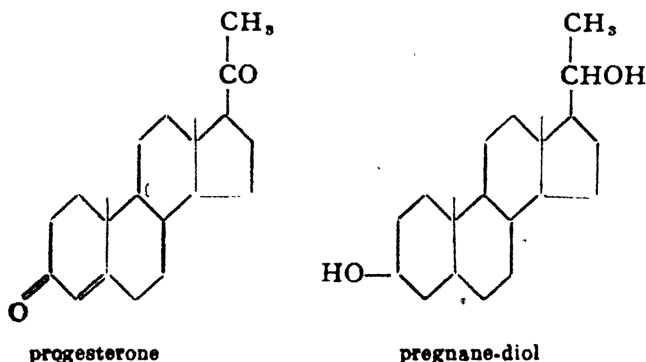
CO

HO—

A

pregnenolone

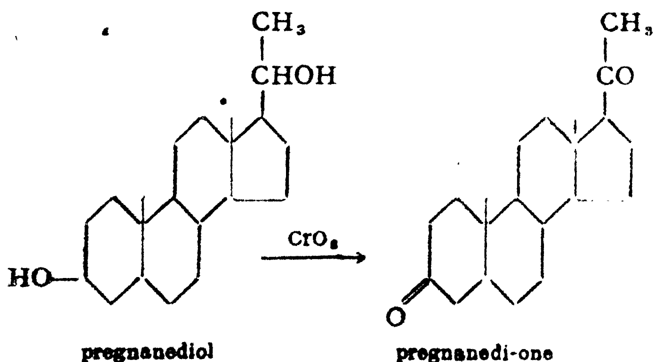
mono-unsaturated diketone related to pregnanediol. The absorption spectrum of the hormone in the ultra-violet indicates the presence of an α-β unsaturated ketone grouping; the X-ray studies also point to the presence of a sterol-like skeleton in the hormone. On the basis of the above considerations, Slotta proposed the following formula for the hormone, which is related to pregnanediol.



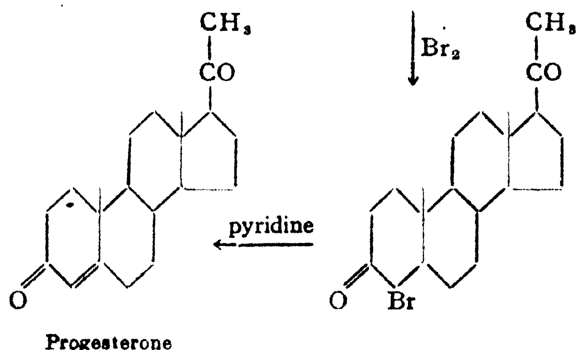
progesterone pregnane-diol

Pregnanediol $C_{21}H_{36}O_2$ is a disecondary alcohol and is a saturated compound. On oxidation, it is converted into a diketone which is reduced with Zn/Hg and HCl to pregnane which is 17-ethyl. One of the OH groups is in the side chain (CH_3–CHOH), as it gives the haloform reaction; the other is in 3 position.

The constitution is confirmed by Butenandt, by converting pregnanediol into progesterone. The steps involved in the conversion are :



pregnanediol pregnanedi-one



Progesterone

In another synthesis, stigmasterol is used as the starting material. The essential steps are indicated below :

$CH_3—CH—CH=CH$
$C_2H_5—CH—CH(CH_3)_2$
HO—

1. acetylation
2. bromination
3. oxidation (O_3)
4. debromination (Zn + acetic acid)

CH_3
$CH—COOH$
HO—

ester + C_6H_5MgBr and dehydration

CH_3
$C=C(C_6H_5)_2$
HO—

(i) ozonisation of the acetate dibromide
(iii) deacetylation

CH_3
CO
HO—
A

pregnenolone

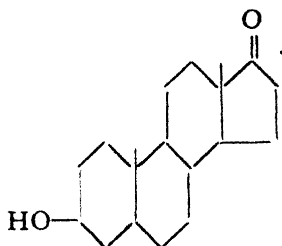
The last compound (A) pregnenolone can be converted into progesterone in two ways: firstly, it is treated with bromine and the dibromide oxidised and subsequently debrominated to give progesterone; secondly, the hydroxy ketone is directly oxidised by Oppenaur's method which is highly selective, to the hormone progesterone. The compound (A) is now available as a by-product of the technical oxidation of cholesteryl acetate dibromide to dehydro-apic-and-rosterone. Also pregnenolone can be obtained from diosgenin and stigamesterol. Diosgenin is treated with $(CH_3CO)_2O$ at 200° to give a product which on oxidation with CrO_3, reduction with Pd and H_2 and hydrolysis is converted into pregnenolone. Stigmasterol is first oxidised to acid which is degraded by the Wieland–Barbier method to pregnenolone. Thus it can serve as a potential source of progesterone.

Androgenic Hormones

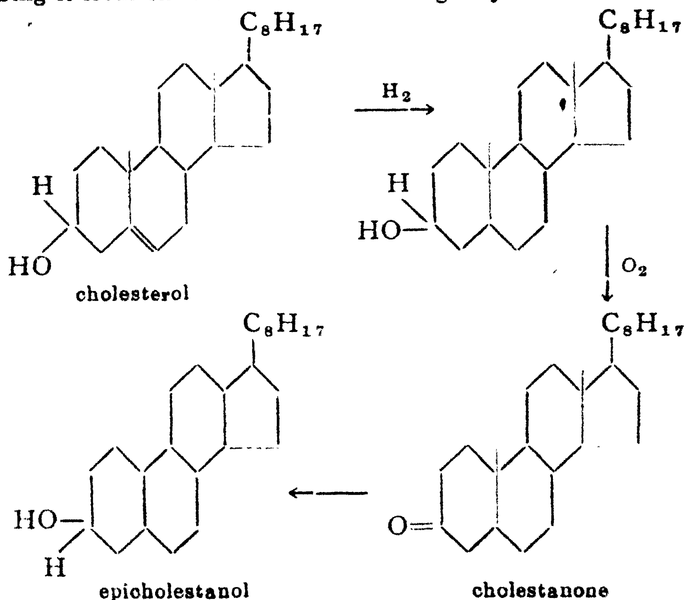
Occurrence. A number of androgenic hormones have been isolated from the urine of animals—male and female and from the testicular extracts. They are responsible for the male characteristics. The two more important ones are androsterone and testosterone.

Isolation of androsterone :—This is present in the urine in the combined form as sulphate. Concentrated male urine is treated with acid and extracted with chloroform. The chloroform layer is repeatedly shaken with aqueous KOH to remove both phenolic and acidic matter. The remaining neutral fraction is treated with Girards reagent T; the ketonic matter is converted into water-soluble compounds; the non-ketonic neutral matter is then removed by extraction with ether. The ketones are then recovered by the hydrolysis of the Girard-reaction product and subsequently purified by chromatography. Androsterone melts at 184°.

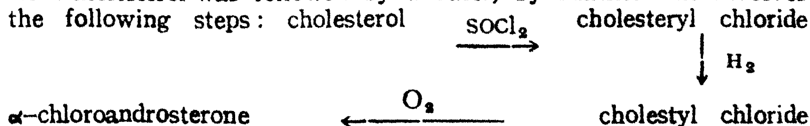
Constitution :—The molecular composition is $C_{19}H_{30}O_2$. It is a saturated compound and forms an acetate or a benzoate and reacts with the Girard T. reagent which is a specific reagent for carbonyl group. Hence it is a saturated hydroxy-ketone; and as it contains eight hydrogen atoms less than a saturated aliphatic ketone, it must contain four rings. On the basis of the above considerations, Butenandt suggested that it had a sterol-like structure and proposed the following formula :



The above structure was confirmed by Ruzicka and others, by preparing it from cholesterol in the following way :—

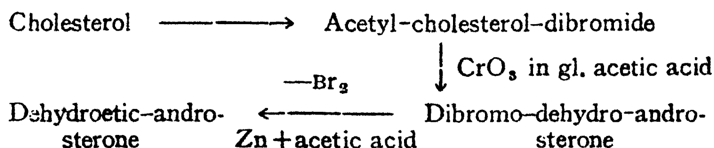


Epi-cholestanol on oxidation with CrO_3 gives androsterone. The yields are very poor, yet it is sufficient evidence for the structure proposed. These conversions are also of great significance as they help to establish a link and relationship between the six hormones and the sterols. This partial synthesis of androsterone from cholesterol was followed by another, by Marker. It involves the following steps : cholesterol $\xrightarrow{SOCl_2}$ cholesteryl chloride

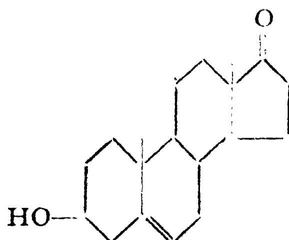


The α-chloroandrosterone is heated with K-acetate and the acetate thus formed is hydrolysed to give androsterone.

Dehydro androsterone :—This is isolated from urine, where it occurs along with androsterone. It has the molecular composition, $C_{19}H_{28}O_2$. Thus it is mono-unsaturated; its exact structure is established from its synthesis from cholesterol.



Hence the structure is :

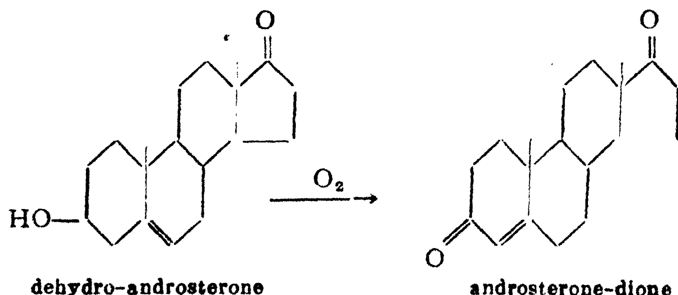


Testo-sterone :— This is found in the testicular extracts. Physiologically it is very potent ; it is a crystalline compound m. p. 154°.

Constitution :—(*i*) It has the molecular formula $C_{19}H_{28}O_2$. It forms acetyl and benzoyl derivatives, thus indicating the presence of an alcoholic OH group.

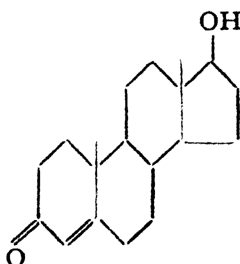
(*ii*) It is very sensitive to alkali like progesterone, which suggests the presence of α β unsaturated keto group, as in progesterone; the absorption spectrum of the hormone, in the ultraviolet confirms the presence of an α β unsaturated keto group.

(*iii*) Lastly, David oxidised testo-sterone to androstene-dione, a compound which is related to and derived from dehydro-androsterone. These relationships help to establish the structure of testo-sterone.



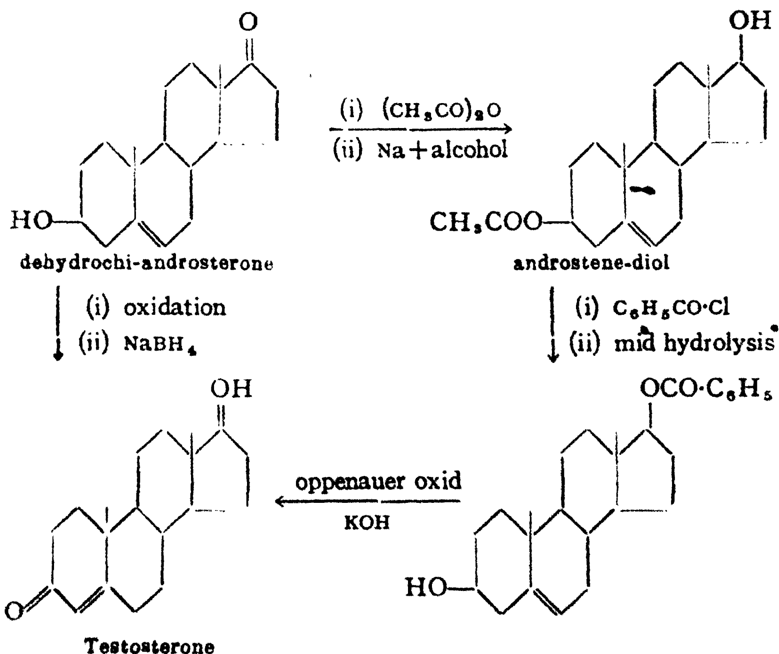
dehydro-androsterone **androsterone-dione**

The latter is obtained from testo–sterone by oxidation; now testosterone contains an alcoholic OH group, which is oxidised to a carbonyl group. Hence testosterone must be represented by :



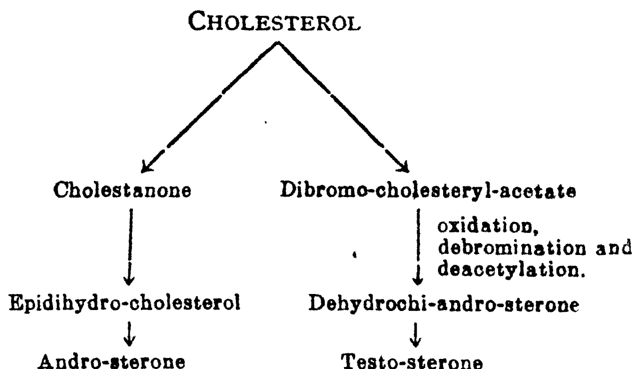
testosterone

The above structure proposed by David has been subsequently confirmed by Butenandt and Ruzicka. These chemists, following similar methods, have converted dehydro–andro–sterone into testosterone. Schematically, the steps involved in the synthesis may be formulated as below :—



Testosterone on progressive "aromatisation" involving final elimination of the 10—CH_3 group as ($COOH \rightarrow CO_2$) gives estradiol.

It is obvious from the foregoing that sex-hormones are structurally very closely related to the most fundamental sterol, cholesterol. Butenandt and Ruzicka have formulated schematically the different relationships existing between the hormones and their probable genesis from cholesterol. The formulation is indicated in the following scheme:—



Adrenal Cortical Hormones

Occurrence and isolation : The cortex of the adrenal glands contain a number of hormones, which are called the "cortex hormones." They are essential for life, and their deficiency produces the typical symptoms of Addison's disease; the deficiency also disturbs the carbohydrate metabolism, and greatly reduces the resistance of the body to cold and shock.

The whole suprarenal glands from oxen are extracted with alcohol or acetone. Adrenaline is then removed by taking advantage of its basic properties. The hormones are hydroxylated and hence relatively more soluble in water and are separated from the fats by distribution between water and hydrocarbon solvents. The mixture of the hormones thus obtained is separated by a judicial combination of the following methods: fractional crystallisation, distribution between solvents and application of the Girard T. reagent. As the hormones which are ketonic show varying degrees of reactivity toward the Girard reagent, a more or less complete separation of the hormones is effected either by the formation or the hydrolysis of the Girard derivatives.

Lastly separation of the hormones has been effectively accomplished by the chromatographic separation of their stable acetates. The hormones, as a class, are very sensitive to both acid alkaline conditions, and hence strong acid or alkaline conditions have to be avoided at any stage of their isolation.

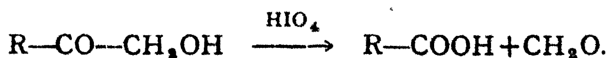
By the application of the above-mentioned methods, Reichstein and others have been able to isolate the following six very important hormones : they are : (*i*) corticosterone, (*ii*) 11-de-dehydro-corticosterone, (*iii*) des-oxy-corticosterone, (*iv*) 17-hydroxy-corticosterone, (*v*) 17-hydroxy-11-dehydro-corticosterone and (*vi*) 17-hydroxy-des-oxy-corticosterone.

Properties of the cortical hormones : All the six hormones are colourless compounds and are dextro-rotatory. They are very sensitive to alkali owing to the presence of α-β unsaturated ketonic group. They reduce readily alkaline silver nitrate solution in the cold ; they readily yield crystalline acetates. Those of them which are oxygenated at C_{11}, exhibit a remarkable green fluorescence in concentrated sulphuric acid solution. They also exhibit a marked effect on carbohydrate metabolism ; they also possess a strong anti-insulin action. Hence they are finding applications in the clinical treatment of Addison's disease and surgical shock.

We shall now discuss the constitutions of the six cortical hormones.

Constitution of corticosterone :—It has the molar composition $C_{21}H_{30}O_4$. The constitution is based on the following considerations :—(*i*) It is very sensitive to alkali and thus resembles progesterone and testosterone. Hence it must contain an $\alpha\beta$ unsaturated ketonic group. This is further confirmed by the results of its absorption spectrum in the ultra-violet.

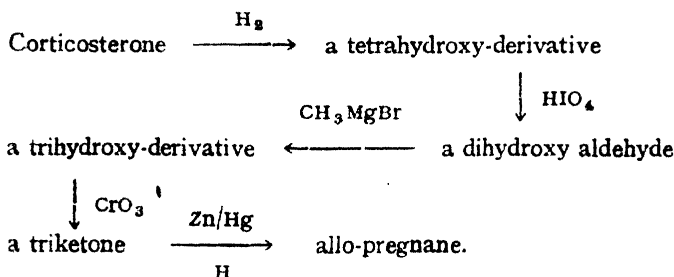
(*ii*) It reduces readily alkaline $AgNO_3$ solution in cold ; this indicates the presence of an aldehyde or a ketol ($—CO—CH_2OH$) group. Oxidation of the hormone with periodic acid yields an acid (C_{20}) and formaldehyde.



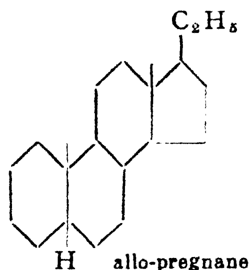
$$R—CO—CH_2OH \xrightarrow{HIO_4} R—COOH + CH_2O.$$

Hence the presence of a ketol group is established.

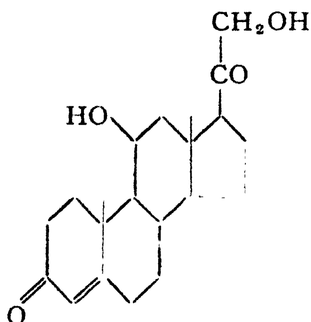
(*iii*) It shows the male hormone activity and hence it must be related to the androgens. The presence of a steroidal skeleton in the molecule is finally established by the following transformation of corticosterone into allo-pregnane :—



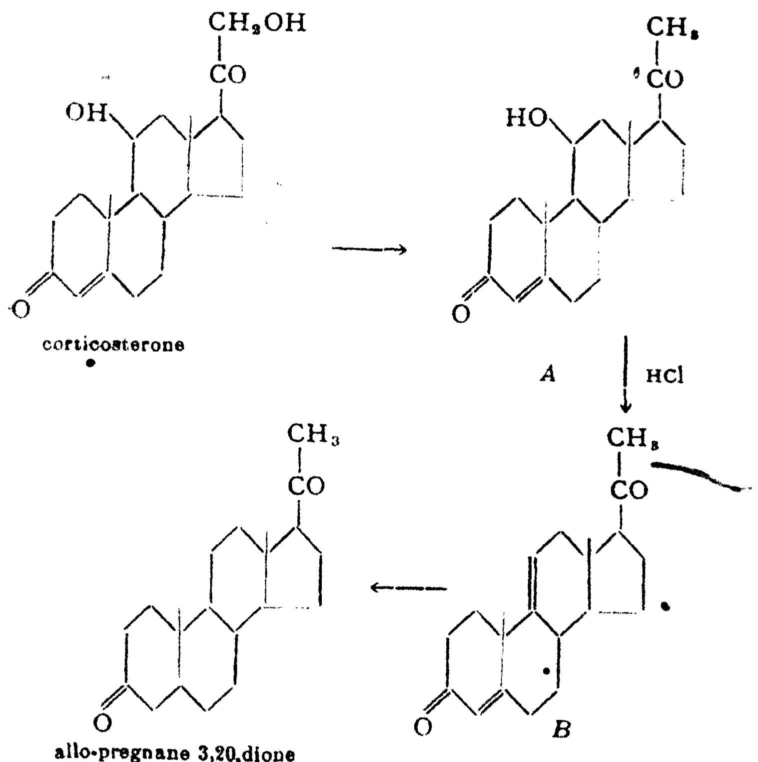
The latter has the structure :



But corticosterone also contains an α-β unsaturated keto group and an α ketol group. Hence it is assigned the structure :



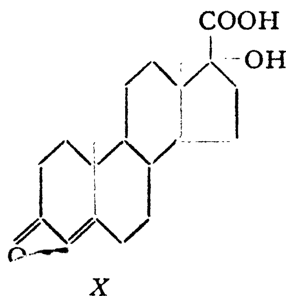
The above formulation especially with regard to the positions of the two ketonic oxygen atoms is established in the following way. Corticosterone is treated with tosyl chloride in pyridine when a mixture of the sulphuryl ester and the chloroketone is formed. The mixture on refluxing with NaI in acetone, gives the iodo-ketone which on reduction gives the methyl ketone A; the latter is identical with 11-hydroxy-progesterone which is dehydrated with HCl to give the compound B. The compound B is subsequently reduced and finally oxidised with CrO_3 to give allo-pregnane-3, 20, dione. Schematically, the changes involved are:



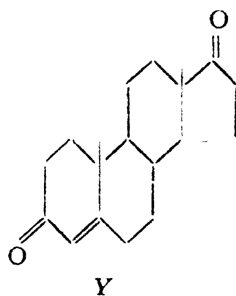
The presence of the hydroxyl group in position 11 is fixed by elimination of other possibilities. It can be acetylated with very great difficulty. CrO_3 can oxidise it to carbonyl group, but the latter does not form any characteristic ketonic derivative. It is shown that the stereo isomer of allopregnane-3,20 dione, which is identical with

dihydro-progesterone, is. a product of degradation and hence cannot have the third oxygen atom in any other position except position 11 or 12; the latter possibility is ruled out by the work of Hoehn and Mason. They prepared a diketonic acid by the degradation of desoxy-cholic acid, which is a 3, 12 diketo derivative. A diketo acid was prepared from corticosterone and was shown to be not identical with the one obtained from desoxy cholic acid. Hence the second keto group in the diketo acid obtained from corticosterone cannot be in position 12; therefore it must have been in position 11. Hence the hydroxyl group must be in position 11.

17-Hydroxy-desoxy-corticosterone : The constitution of this compound is established by converting it by suitable reactions into steroids of known constitution. Thus this hormone on oxidation with periodic acid is converted into the known 17 (β)-hydroxy-3-keto-Δ^4-acetio-cholenic acid (X), while CrO_3 oxidation of the hormone yields the known Δ^4- androstene-3, 17, dione (Y).

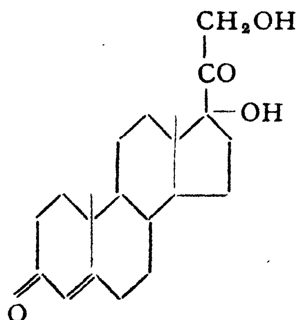


X

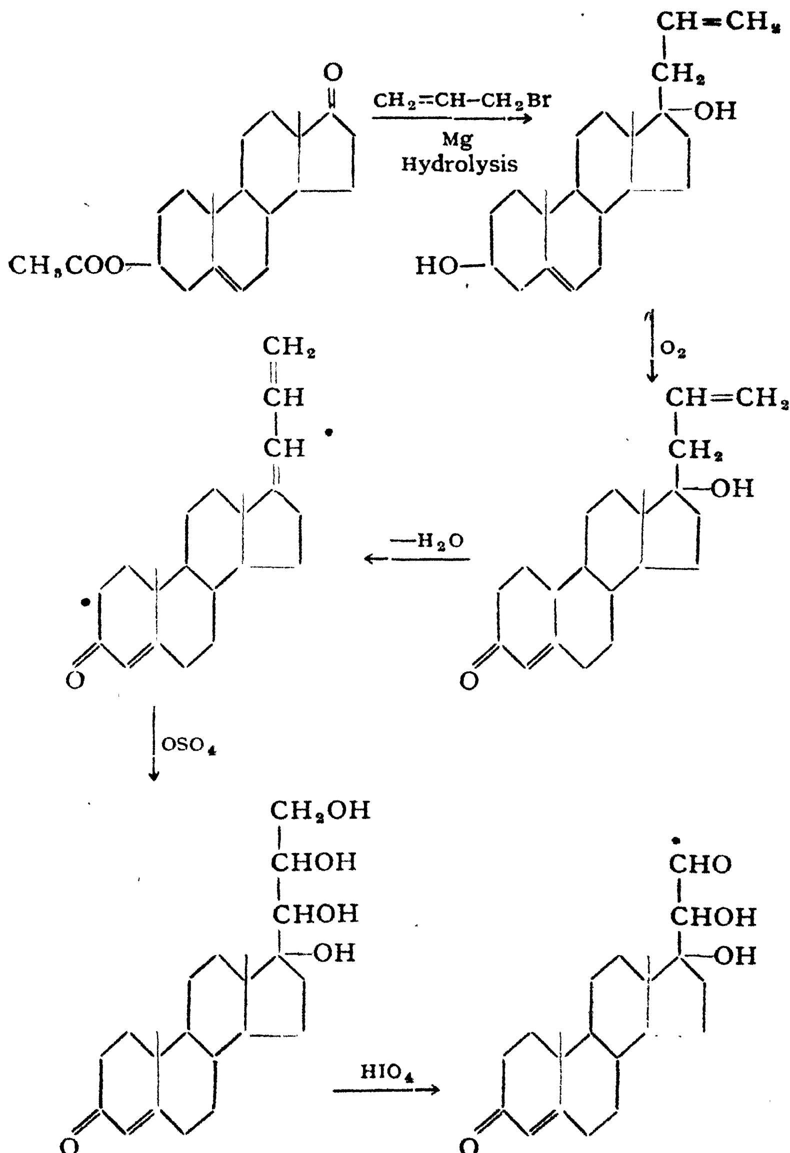


Y

The above conversions are satisfactorily accounted for, on the basis of the following structure for 17(β) hydroxy–desoxy–cortico–sterone :

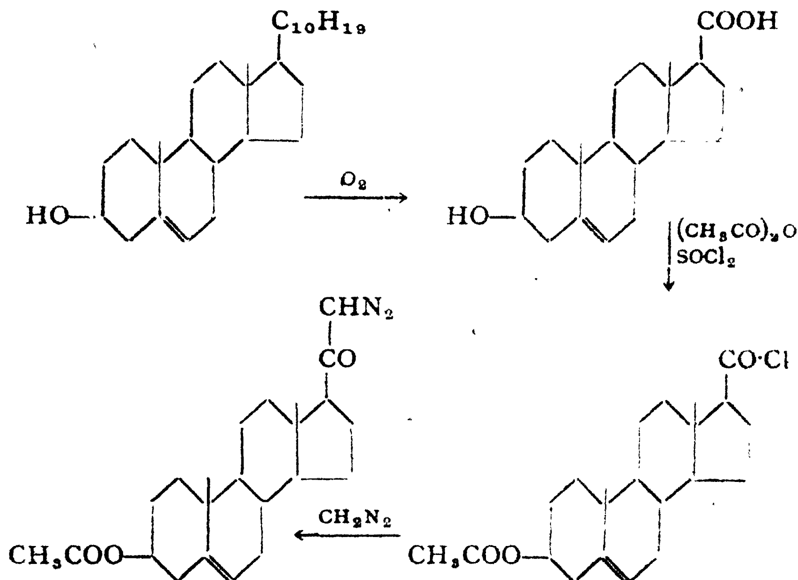


This structure is further confirmed by its partial synthesis from dehydro-andro-sterone-acetate, which involves the following steps :

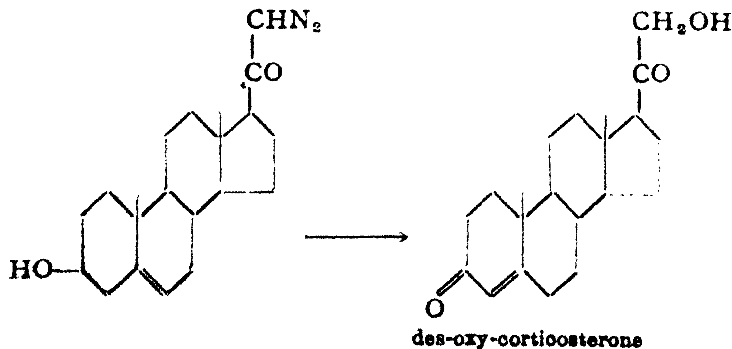


The α-hydroxy-aldehyde on heating with pyridine is converted into the α-ketol-group thus giving a compound identical with the hormone.

Des-oxy-corticosterone. The constitution of this hormone is established by a partial synthesis of the same, from stigmasterol. The essential steps involved are outlined below :—

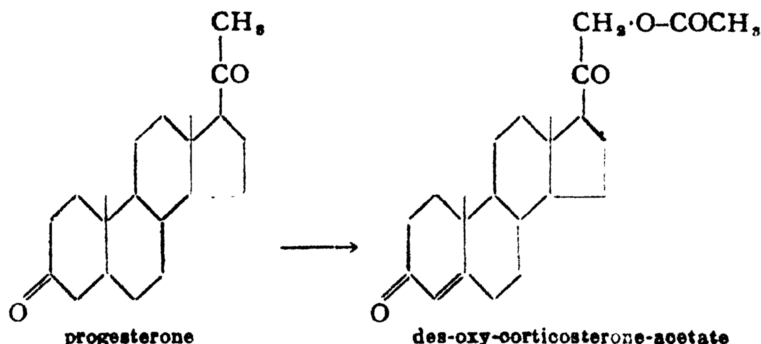


The last compound, on alkaline hydrolysis in the cold, gives the corresponding hydroxy-diazo-ketone; the latter on Oppenauer-oxidation is converted into a compound which on treatment with dil·H_2SO_4 gives the hormone : des-oxycorticosterone.

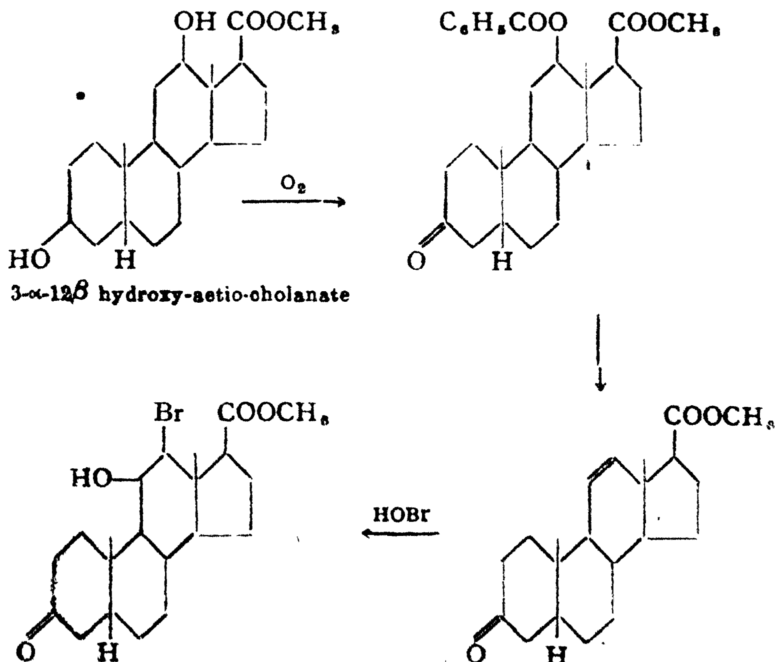


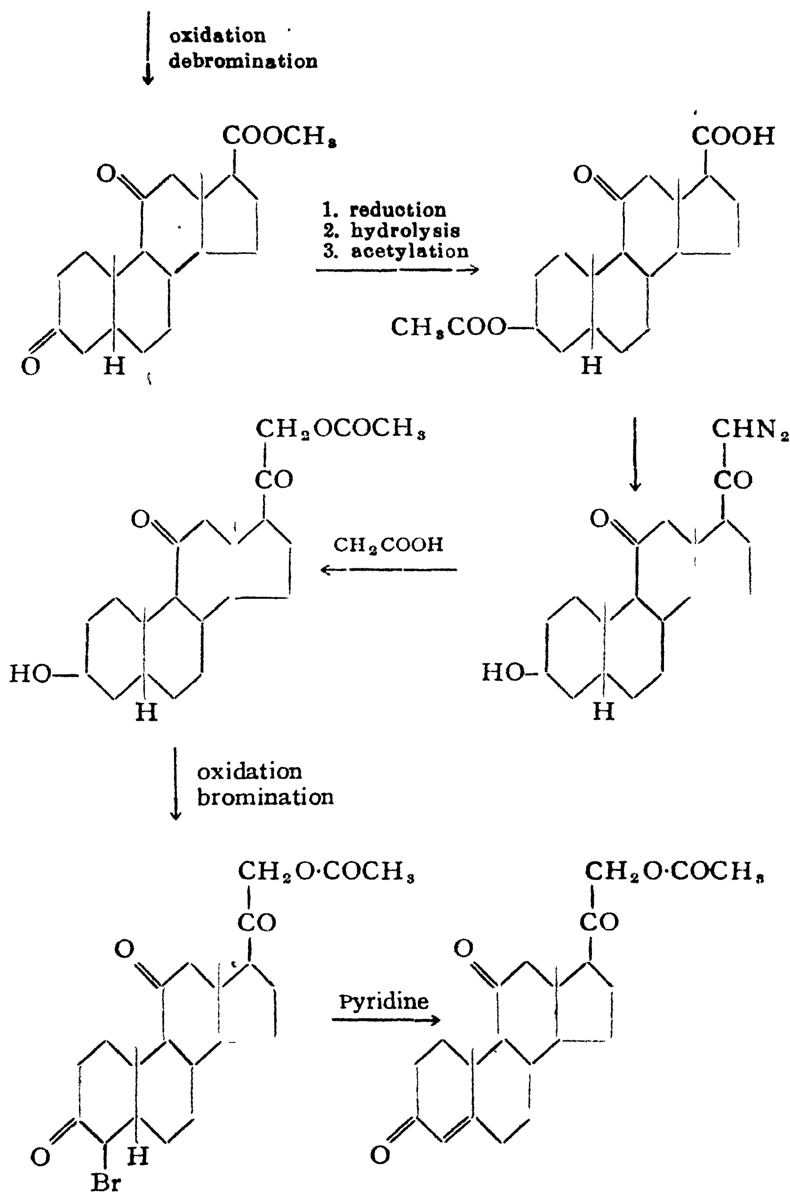
des-oxy-corticosterone

The conversion of progesterone into des-oxy-corticosterone acetate, by oxidation with leadtetraacetate, is also in agreement with the structure described above:



De-hydro-corticosterone. The constitution of this adrenal hormone is established by a synthesis starting from a 12-oxygenated bile acid or its degradation product. The synthesis involves several stages which are outlined below:

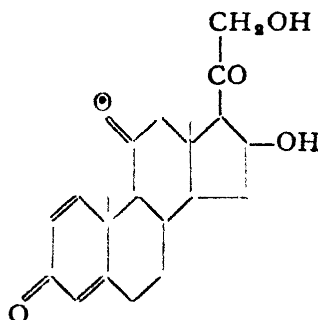




Dehydro-corticosterone-acetate

The above synthesis has a twofold significance. It establishes conclusively that in the case of the oxygenated steroidal hormones, the oxygen atom in ring C is situated in position 11. Further it suggests that all the closely related adrenal cortical hormones can be ultimately derived from the relatively more readily available sterols or the bile acids and their derivatives.

Cortisone (17 hydroxy–11 dehydro–cortisterone) is the most active of these hormones. It is obtained from progesterone ; at some stage, microbial oxidation is utilized. Microbiological oxidation with the Rhizopus species can be used to oxidise C_{11} to C_{11}–OH and to dehydrogenate the ringone in the steroids ; thus cortisone is converted into 1 dehydro cortisone.



CHAPTER VIII

PROTEINS AND POLYPEPTIDES

Introduction :—Proteins are highly complex nitrogenous organic compounds of high molecular weights (30,000 or higher). They are widely distributed in both the animal and plant kingdom ; they form the constituents of all livings cells. They, thus, serve as the structural material for the tissues of plants and animals. They are absolutely essential to life processes. The plants can build up their specific proteins from the inorganic nitrates, ammonium sulphate, carbon dioxide and water ; the animals, on the other hand, cannot synthesise them from such inorganic sources. Hence, proteins constitute one of the important and essential food-stuffs of animals.

The chemistry of proteins is still shrouded in mystery. Despite the large amount of work, both analytical and synthetical, the architecture of the highly complex protein molecule has not been unravelled. The chemical investigation of proteins has precented almost insuperable difficulties. They are highly complex molecules colloidal in nature, in whose case, the usual methods of isolation fail. They decompose before they melt and hence, the ordinary criteria of purity cannot be applied to them. They cannot be crystallised, nor can they be purified by distillation. They are also extremely sensitive to heat and chemical reagents, *e. g.* acids, alkalies and salts. Recently a few proteins, *e. g.* hemp albumin, serum albumin and ovalbumin have been obtained in crystalline forms.

General composition and behaviour :—Hundreds of proteins are known in nature, differing widely in their physical and chemical properties, yet in their composition they are remarkably *uniform*. They contain the following few elements only : carbon, hydrogen, nitrogen and oxygen ; and in smaller proportions sulphur, phosphorus, iron and copper. (*Fe* as a constituent of *hæmoglobin* and *Cu* of *hæmocyanin).* Iodine is present in a few proteins as thyreo-globulin ; further the relative proportions of these elements vary within very narrow limits :—

C	50·5	to	54·6	per cent
H	6·5	,,	7·3	,,
N	15·0	,,	17.6	,,
O	21·5	,,	23·5	,,
S	0·5	,,	2·2	,,
P	0·42	,,	0·85	,,

Also, in another fundamental respect, the proteins are similar; on hydrolysis, they all yield a mixture of α-amino acids. From the hydrolysis of hundreds of proteins, so far only *twenty-three* different α-amino acids have been isolated. These latter, thus, appear to be the 'building blocks' out of which, the complex hundreds of proteins have been built up.

Proteins are highly colloidal in nature, and do not diffuse through animal membranes. Many are *l*-rotatory they belong to the *L* family insoluble in water, but soluble in saline solutions. The colloidal property has been utilised in their separation from the electrolytes (dialysis). Proteins can be readily *coagulated* by heat. Coagulation is the total effect produced by the action of heat, acid, alkali etc. on the proteins, which consists of denaturation and precipitation. Apparently denaturation involves some chemical changes in the protein molecule. A denaturated protein is insoluble in water at its iso-electric point; the protein is precipitated following the denatura-tion. Coagulation is an irreverssible change.

Proteins are amphoteric. They are built of chains of α-amino acids. Some of the latter acids carry free CO_2H and NH_2 groups so that a few such groups lie scattered at different points along the chain; this makes the molecule amphoteric. A protein built up of neutral amino acids will have but one free NH_2 and one free CO_2H at either end of the chain; but these groups will not exert any influence, because of the molecular weight of the whole protein molecule.

The protein coagulates best at a certain pH value which is known as its '*iso-electric point*'. The iso-electric point for many proteins is pH 4·7. This point corresponds to the minimum values of viscosity, conductivity and osmotic pressure for the protein.

The iso-electric point in pH units is a specific value for a protein, at this point the protein does not move in the electric field. At a pH lower than this one, the protein exists mainly as the cation and moves towards the cathode. At higher pH values than the iso-electric point the proteins exist as the anion and move towards the anode. This movement in an electric field is known as electrophoresis and methods based on this principle have been developed for the separation of proteins.

The precipitation of the proteins by the addition of a neutral salt must be differentiated from the coagulation of the protein by heat or precipitation of the protein by the addition of alcohol or acetone. The precipitation of some proteins by neutral salts has been found to be *reversible* and has been used by Hofmeister for the isolation of some of the proteins. The proteins can be separated partially by fractional precipitation with $(NH_4)_2SO_4$; the concentration of the salt required for precipitation is definite for each protein. The temperature of coagulation is also specific. The effect of the neutral salt has been ascribed to the specific influence of the ions of the salt.

Another important property of proteins is their 'protective' action; when present in small quantities, they prevent colloids from being precipitated by electrolytes. For the same electrolyte, the amounts of the different proteins are found to vary. Zsigmondy has evolved a standard value called the '*gold number*' to measure the protective action of a protein. The gold number has been defined as the number of milligrammes of a protein which is just insufficient to protect 10 c. c. of a standard colloidal gold solution from precipitation by 1 c. c. of a 10 per cent solution of sodium chloride. This number is a specific one for each protein.

Methods for the detection of proteins :—The proteins, as a class, give certain reactions which can be used for the detection of the presence or absence of a protein. However, no single one of these reactions is reliable, as such a reaction is also given by other complex organic compounds. But still they have been used to settle cases of doubt. These reactions have been subdivided into *(a) precipitation reactions* and *(b) colour reactions.*

The precipitation reactions are due to the colloidal nature of the protein and thus give a more reliable information as to the presence of the protein. The precipitation reactions are .

(i) Coagulation test :—Proteins are precipitated by the application of heat, from faintly acid solutions *e.g.* the boiling of an egg.

(ii) Salt formation test :—The addition of the salts of heavy metals *e.g.* mercuric chloride, basic lead acetate, copper sulphate, ferric chloride etc. precipitates the proteins very readily.

(iii) Heller's test :—In this test, the precipitation of the protein is effected by concentrated nitric acid. This test is very common, and used as a delicate test for albumen in urine.

(iv) Other reagents :—The usual alkaloidal reagents, *e.g.* phospho-tungstic acid, tannic acid, picric acid etc. also precipitate almost quantitatively the proteins which are on the acid side of the isoelectric point from their solutions. This is probably due to the latter's slightly basic properties.

The colour reactions depend on the occurrence of specific chemical groups in the protein molecule ; and hence they may be given by non-protein compounds containing the specific groups. The colour reactions are :

(i) Millon's reagent :—A solution of mercury in nitric acid which contains Hg_2'' (ous) and Hg'' (ic) nitrates and nitrous acid gives with proteins, a *pink* colouration on boiling. (This test is given also by compounds containing phenolic groups. In the case of a protein it is probably due to the tyrosine group).

(ii) Xantho-protein test :—A protein solution, on treatment with concentrated nitric acid, turns *yellow* as a result of formation of yellow nitro compounds. On the addition of excess of ammonia the colour changes to *orange*. This test is given by proteins with tyrosine groups.

(iii) Biuret test :—This test is given by compounds with the *CO—NH* grouping *e.g.*, biuret. The test consists in treating the compound with copper sulphate solution containing a trace of alkali when *violet* colour is developed. It has been employed to distinguish between proteins and the partially hydrolysed products *e.g.* peptones and albumoses.

(*iv*) *The ninhydrin test* :—When a protein solution after hydrolysis is heated with ninhydrin (triketo-hydrindene) a *blue* colouration is obtained. This test is given by α-amino acids only.

(*v*) *The iodine test* :—Proteins, when mixed with iodine in alcohol or potassium iodide solution, develop yellow colour.

Classification :—A rational and scientific classification of the proteins has not been so far achieved. The molecular formulas of the proteins are unknown. The exact number of the α-amino acids, their modes of combination etc. are also undecided. Hence, a pure chemical basis for their classification is not yet available. However, an arbitrary classification based on some physical properties has been adopted; according to it, the proteins are classified as : (*a*) **Fibrous proteins** and (*b*) **Globular proteins.** All the modern physical technique tends to confirm this division. This classification is functional; the function of the fibrous proteins is mainly architectural. They are probably the linear condensation products of α-amino acids. The chains are held together by multiple H-bonding. Chemically, they are inert. The globular proteins, on the other hand, constitute the solid portion of the proto-plasm; They appear to be more highly branched and cross-linked than the fibrous proteins. Chemically they are active. They are further subdivided into :—

(*i*) SIMPLE PROTEINS :—On hydrolysis, they give a mixture of α-amino acids only *e.g.*, albumin, globulin.

(*ii*) CONJUGATED PROTEINS OR PROTEIDS :—They are built up of a simple protein and a non-protein group, called the prosthetic group, which is usually an acidic group. On hydrolysis, the conjugated proteins give a mixture of α-amino acids and at least one of the following types of compounds : carbohydrates, phosphoric acid and nucleic acids. Thus, we have gluco-proteins, phospho-proteins, nucleo-proteins and lecithins.

(*iii*) DERIVED PROTEINS :—They represent the decomposition products of more complex proteins and are subdivided into (*a*) primary and (*b*) secondary derived proteins. The primary derived proteins are proteins, meta-proteins and coagulated proteins; the secondary derived proteins are proteoses, peptonesa nd the peptides.

Molecular weights of proteins :—The problem of the molecular weights of the protein molecule has still remained unsolved. It has not been possible to obtain a natural protein in a pure crystalline form. So far only *empirical* formulas have been assigned to a few proteins. Their exact molecular weights have not been ascertained. The usual physico-chemical methods of determining the molecular weights fail in the case of proteins. However, *minimum* molecular weights of some proteins have been obtained by purely chemical methods. Hæmoglobin contains 0 to 4 per cent of iron ; a molecule of hæmoglobin must contain at least *one* atom of *Fe*. Hence, the minimum value for the molecular weight is 14,000 ; the value calculated on the basis of absorption of oxygen comes to 16,000 ; practically the same value is indicated by osmotic pressure measurements of Hufner and Gauss. Still we have to remember that a chemically pure specimen of hæmoglobin has not been prepared.

Recently, Svedberg has developed an *ultra-centrifuge* method for determining the molecular weight of proteins. It depends on the rate at which the colloidal protein particles settle under the tremendous forces exerted by an ultra-centrifuge. The centrifugal force exerted is equivalent to 400,000 times the force of gravity. The sedimentation rate is proportional to the size of the particles *i. e.* the molecular weight and shape ; hence the molecular weight can be approximately computed. The molecular weights determined by these methods vary from 16,000 to 35,000. Proteins, thus, appear to be the *largest atomic aggregates constituting individual molecules.*

Still more recently, molecular weights of proteins have been determined by the electro-phoretic method developed by Tiselius. The molecular weights as determined by this method appear to be multiples of 35,000 ; the figures for the molecalar weights of many proteins are divisible by this number. This may be an indication of the existence of a repeating unit of this size, in the native protein molecule.

Structure of the proteins :—Our present knowledge of the structure of a protein molecule is based on the results of analytical methods and the special synthetic methods developed by Fischer. The usual analytical methods consist in breaking down the complex

molecule into a number of simple and readily identifiable products. For this purpose, the standard reactions employed are: (*a*) hydrolysis, (*b*) oxidative degradation and (*c*) reductive degradation.

The products of oxidation of the protein molecule usually consist of a complex mixture of fatty acids, aldehydes, ketones, nitriles, HCN and benzoic acid. The mixture is difficult to separate and hence no useful clues regarding the structure have been possible. Similarly with reduction reactions also, the products are highly complex, non-separable mixtures; and hence these reactions have not at all been fruitful in the elucidation of the structural relationships of the proteins. However, results of hydrolysis have given very important clues to the solution of the structural problem of the proteins. In fact, the modern knowledge regarding the structure of proteins rest chiefly on the results of hydrolytic decomposition methods. Hydrolysis of a protein is effected in one of the following ways.

Acid hydrolysis: The protein is boiled with 20·5% HCl or with $8N \cdot H_2SO_4$. The hydrochloric acid hydrolysis is prefered, if the subsequent separation of the α-amino acids is to be effected by the Fischer's method. The Dakin's procedure for the separation of the α-amino acid mixture, requires a mixture free from mineral acid; sulphuric acid can be effectively removed after the hydrolysis, by precipitation with $Ba(OH)_2$. Hence sulphuric acid is used as the hydrolysing agent, if Dakin's process is to be subsequently adopted for the separation of the α-amino acids.

The yield of the α-amino acids is very high; but there are certain disadvantages: (*i*) tryptophane is completely destroyed; similarly the hydroxy-amino acids, serine and threonine are also attacked: (*ii*) cysteine is oxidised to cystine. This is however prevented by the addition of tin to the acid used for hydrolysis; (*iii*) the hydrolysate is coloured owing to the formation of humin; which also is avoided by the addition of tin. Recently a new acid mixture has heen proposed as the hydrolysing agent; it is an aqueous solution containing 20% HCl and 50% formic acid.

Alkaline hydrolysis: This consists in boiling the protein with dilute NaOH or with $4N \cdot Ba(OH)_2$. Complete hydrolysis is achieved within a period of ten hours. In this hydrolysis, the decomposition

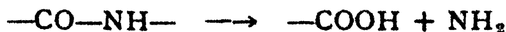
of trypto-phane is completely avoided, and a quantitative yield of this α-amino acid is obtained. Another great advantage is that the hydrolysate obtained is colourless and free from humin. However, the alkaline hydrolysis possesses the following practical disadvantages; (*i*) the amino acids formed, undergo racemisation; (*ii*) some of the amino acids are deaminated; (*iii*) arginine is converted into ornithine and urea; (*iv*) both cysteine and cystine are destroyed by alkali.

Enzymatic hydrolysis: The protein is degraded by the agency of a specific enzyme. The most typical and common proteolytic enzymes *i.e.* proteases are: (*i*) pepsin, present in the stomach, (*ii*) trypsin present in the pancreatic juice and (*iii*) erepsin, present in the intestines. The degradation of the protein molecule takes place stepwise and intermediate compounds of decomposition have been actually isolated.

Proteins → Proteoses, peptones → polypeptides → α-amino acids. This partial or stepwise hydrolysis may be used to determine the nature of the protein molecule. The peptide fractions formed, can be isolated and their structures established, thus giving a clue to the structural units or the structural pattern present in the complex molecule. The animal enzymes act in acid or alkaline medium only. Thus pepsin acts in acid medium only; trypsin acts in alkaline medium while papain–an enzyme from plants acts in neutral medium. Finally, no single enzyme will effect the complete hydrolysis of a protein to a mixture of α-amino acids.

However, the progress of hydrolysis of the protein is very slow; there is also another disadvantage; the enzymes used are themselves proteins and the hydrolysates therefore are likely to be contaminated with residues from the split enzyme molecules. The enzymatic hydrolysis is however free from recemisation and other secondary reactions. Finally, no single enzyme will effect the complete hydrolysis of a protein to a mixture of α-amino acids.

The final product of acid, alkaline or enzymatic hydrolysis is a mixture of α-amino acids. The proteins contain the peptide linking —CO—NH— which on hydrolysis forms equivalent amounts of carboxyl and amino groups:



$$—CO—NH— \longrightarrow —COOH + NH_2$$

Hence the progress of hydrolysis may be followed by one of the following methods: (*i*) Formol-titration method, (*ii*) von Slyke's method and (*iii*) Linder-strom Lang method.

Isolation of the amino acids

The product of hydrolysis of a protein is thus a highly complex mixture of α-amino acids, which are non-volatile and hence difficult to separate. Many methods have been developed from time to time, to secure an effective and quantitative separation of the amino-acids. They are based on different principles: thus we have (*i*) Fischer's method based on the fractional distillation of the esters, (*ii*) Dakins method involving differential solubilities in different solvents, (*iii*) The paper-partition chromatographic method of Synge, (*iv*) The electrical transport method depending on the presence of electrical charges on the colloidal protein molecules, (*v*) Ion-exchange methods and (*vi*) Precipitation methods.

Fischer's method: The protein is hydrolysed with hydrochloric acid; on cooling the hydrolysate; the insoluble chloride of glutamic acid is precipitated out. After removal of the glutamic acid, the excess of HCl is removed under reduced pressure. The residue is then esterified with absolute ethanol and HCl gas. The glycine ester is insoluble and is removed by centrifugation. The chlorides of the remaining α-amino acid esters, are treated with alkali and then distilled in vacuo. The following are the fractions (ester of the acids) collected with their probable composition.

Fraction I (60°C. 10 mm.) glycine, alanine, leucine, proline.

Fraction II (100°C, 10 mm.) valine, leucine, proline.

Fraction III (100°C, 0.5 mm.) leucine, proline.

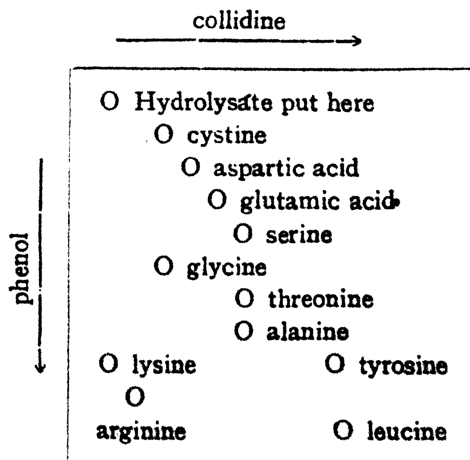
Fraction IV (180°C, 0.5 mm.) phenylalanine, serine, aspartic acid.

The esters thus separated are hydrolysed to the free α-amino acids, which are further purified by fractional crystallisation or by means of specific reagents. The method, however involves great losses and the results are not quantitative. This necessitates the use of large amounts of the original protein matter.

Dakin's methods: It is based on the principle of distribution between two suitable solvents; the amino acids are distributed between

water saturated with butyl alcohol and butyl alcohol saturated with water. The mono-amino-mono-carboxylic acids and proline are completely extracted; the individual acid can then be separated by fractional crystallisation or fractional distillation of their esters. The diamino-mono carboxylic acids which are basic and the mono-amino-dicarboxylic acids which are acidic, remain in the aqueous layer. The former ones are then separated by precipitation with phospho-tungstic acid. This method possesses the advantage that the amino-acids which are optically active do not suffer any racemisation during the process of separation.

Partition Chromatographic method : The α-amino acids are partitioned between water and organic solvents which are immiscible with, but partly soluble in water, *e.g.* butanol, phenol, collidine etc. The mixtures of α-amino acids (the protein hydrolysates) are adsorbed on starch, silica gel, or filter-paper strips, and the organic solvents saturated with water are allowed to flow through the adsorbent. The amino acids are extracted by the solvents at different rates and hence are separated from one another. Further separation may be effected by the application of a second solvent. If the filter-paper is used as the solid phase (the adsorbent), and the direction of the flow of the two solvents is made to differ by 90°, the mixture of amino acids is resolved into spots which are made visible by the ninhydrin reagent. Each spot consists of a single amino acid, and thus the separation of the mixture is quite complete.



The intensity of the ninhydrin spots can be measured spectrophotometrically by the determination of the transmission of the filter-paper; thus even a quantitative determination of the various amino acids is made possible. This is the paper-partition chromatography based on extraction by a *descending* solvent. Recently, paper chromatography by capillary ascent has been developed and is highly preferable. Another modification is to subject the acetamido-amino acids to partition chromatography; Sangers has employed the dinitro-diphenyl derivatives for *the same* purpose with great advantage.

The practical advantages of the above technique are many; some of the important ones are: (*i*) a very small quantity of the protein hydrolysate is required; (*ii*) the separation of the mixture of the amino acids can be made both qualitative and quantitative; (*iii*) it has provided a proof that nor valine and nor-leucine do not exist in natural proteins; (*iv*) the presence of hydroxy-glutamic acid in casein could not be confirmed; it is shown to be a mixture of aspartic acid with other substances.

The electrical transport method: An electric current is passed into the protein hydrolysate at pH 5·5. The *acidic* amino acids migrate to the anode, and the basic ones travel to the cathode; the neutral ones remain in the central compartment thus a separation of the amino acids in the hydrolysate is made possible.

Ion exchange methods: Recently, a new technique involving the use of synthetic resins for the separation of the amino acids has been developed. Thus the basic diamino-mono carboxylic acids are removed from the hydrolysate by the use of acid resins which consist of phenol sulphonic acids. In a similar way, the acidic dicarboxylic mono amino acids are separated by the use of basic resins. Lastly the remaining neutral mono amino—mono-carboxylic acids are removed and subsequently concentrated by a strongly acid resin.

Precipitation methods: These methods are highly specific, the individual acids are thus quantitatively precipitated by the use of specific reagents. Thus histidine is quantitatively precipitated from a mixture by the addition of 3, 4-dichloro-benzene-sulphonic acid; tryptophane is isolated from the protein hydrolysate by precipitation

ith $HgSO_4$. The Reinecke's salt: $NH_4[Cr(CNS)_4(NH_3)_2]$ precipitates both proline and hydroxyproline, while NH_4 rhodanilate precipitates only proline.

So far some twenty-three α-amino acids have been isolated from several protein hydrolysates, by the application of one of the above mentioned methods. These results thus clearly indicate that several hundred of proteins found in nature are built up ultimately of twenty-three building blocks in the form of these α-amino acids.

Classification of α-amino acids: The twenty-three amino acids have been classified into four divisions based on the fundamental structural differences. Thus we have:

1. Aliphatic amino acids:
   (a) *acidic:* mono-amino-dicarboxylic acids.
   (b) *basic:* diamino-mono-carboxylic acids.
   (c) *neutral:* mono-amino-mono-carboxylic acids.
2. Aromatic amino acids.
3. Hetero-cyclic amino acids.
4. Sulphur containing amino acids.

Aliphatic amino acids

Acidic: Mono amino-di-carboxylic acids:

Aspartic acid $HOOC \cdot CH_2—CH(NH_2)—COOH$

Glutamic acid $HOOC \cdot CH_2—CH_2—CH(NH_2)—COOH$

These are distinctly acidic, and form barium or calcium salts which are sparingly soluble in aqueous alcohol.

Basic: di-amino-mono-carboxylic acids:

Lysine $H_2N(CH_2)_4—CH(NH_2)—COOH$

Arginine $$\begin{matrix} H_2N \\ HN \end{matrix}\!\!>\!C—NH—(CH_2)_3—CH(NH_2)—COOH$$

Neutral: mono amino-mono-carboxylic acids:

Glycine H_2N-CH_2-COOH

Alanine $CH_3-CH(NH_2)COOH$

Serine $HOCH_2-CH(NH_2)-COOH$

Threonine $CH_3 \cdot CHOH-CH(NH_2)-COOH$

Valine $(CH_3)_2\ CH-CH(NH_2)-COOH$

Nor-leucine $CH_3-(CH_2)_3-CH(NH_2)-COOH$

Leucine $(CH_3)_2CH-CH_2-CH(NH_2)-COOH$

Iso-leucine $\begin{matrix} C_2H_5 \\ CH_3 \end{matrix} \Big> CH-CH(NH_2)-COOH$

Ornithine $H_2N-(CH_2)_3-CH(NH_2)-COOH$

Aromatic amino acids

Phenyl alanine $C_6H_5-CH_2-CH(NH_2)-COOH$

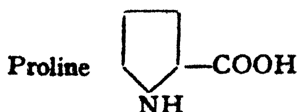
Tyrosine $HO-\langle\ \rangle-CH_2-CH(NH_2)-COOH$

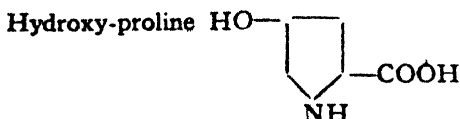
Iodo-gorgoic acid $HO-\underset{I}{\overset{I}{\langle\ \rangle}}CH_2\text{-}CH(NH_2)\text{-}COOH$

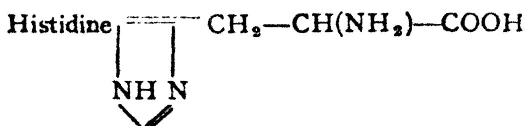
Thyroxine $HO\text{-}\underset{I}{\overset{I}{\langle\ \rangle}}\text{-}O\text{-}\underset{I}{\overset{I}{\langle\ \rangle}}\text{-}CH_2\text{-}CH(NH_2)\text{-}COOH$

Heterocyclic amino acids

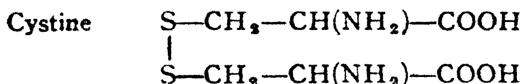
Tryptophane $\underset{NH}{\langle\ \rangle}-CH_2-CH(NH_2)-COOH$

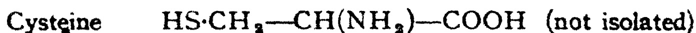


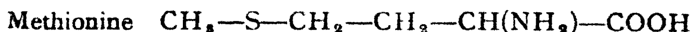




Sulphur containing amino acids





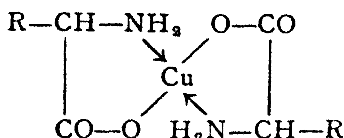


N. B.—Later researches have shown that hydroxy-glutamic acid is a mixture of aspartic acid and other compounds.

The extensive nutritional research has revealed that the presence of the following amino-acids, is essential, for the growth of living organism. The absence of any one of them, may even cause death of the organism. They are thus indispensable because they cannot be synthesised in the body by the organism and are necessary for the required protein diet. Hence they are called the "essential acids." They are: (1) Lysine, (2) Tryptophane, (3) Histidine, (4) Leucine, (5) Phenylalanine, (6) Iso-leucine, (7) Threonine, (8) Methionine, (9) Valine, (10) Arginine and (11) Aspartic acid.

General methods of synthesis of α-amino acids— A number of methods of synthesising the amino acids have been developed. The isolation of the free amino acid from the reaction mixture is

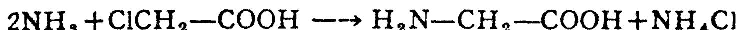
effected through its copper salt. The latter is a deep-blue compound which can be crystallised from the solution; it is probably a co-ordination complex compound, and the structure of the copper compound of the amino acid, $R—CH(NH_2)—COOH$, is :



The copper compound is then decomposed into copper sulphide and the amino acid, by passing hydrogen sulphide into the hot solution of the copper salt. The synthetic amino acids thus obtained are racemic. They are formylated or benzoylated and then resolved by the use of an active base like quinine or the NH_2 group may be blocked by an optically active acid residue and the derivative thus obtained then separated by fractional crystallisation.

The following are some of the important methods of synthesis of the α-amino acids :

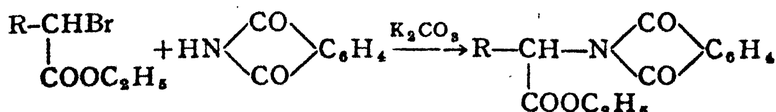
1. FROM α-HALOGENATED ACIDS AND AMMONIA:—This is the first method used for the synthesis of the α-amino acid, glycine.



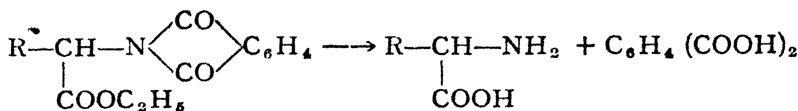
$$2NH_3 + ClCH_2—COOH \longrightarrow H_2N—CH_2—COOH + NH_4Cl$$

A large amount of ammonia is necessary; even then the yield is poor and the product difficult to purify owing to the simultaneous formation of compounds like $NH(CH_2—COOH)_2$ and $N(CH_2—COOH)_3$.

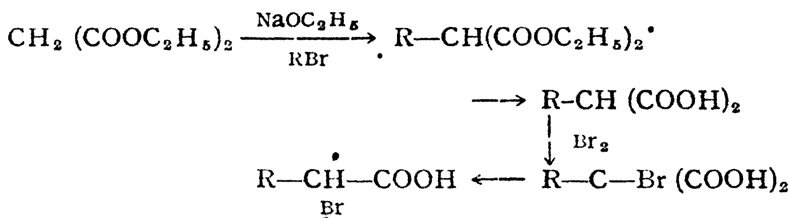
The formation of the by-products is avoided by a slight modification which consists in the application of the Gabriel synthesis. The α-halogenated acid ester is made to interact with the potassium salt of phthalimide; or the α-halogenated acid ester is refluxed with phthalimide in acetone solution, in presence of anhydrous K_2CO_3 :



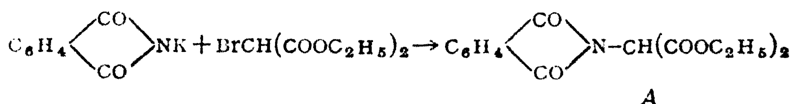
The product is then hydrolysed to the α-amino-acid and phthalic acid, by refluxing it with alcoholic KOH or with NH_2—NH_2 (85%).



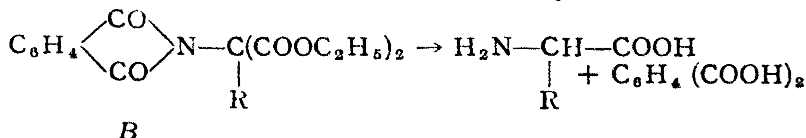
The yields of the amino acids, are quite satisfactory. The α-halogenated acids required in the above synthesis are obtained from malonic ester according to the following scheme :



2. SORENSEN'S METHOD :—This method consists of an ingenious combination of Gabriel's synthesis and malonic ester synthesis. Bromo-malonic ester is made to interact with the potassium salt of phthalimide to form phthalimido-malonic ester A.

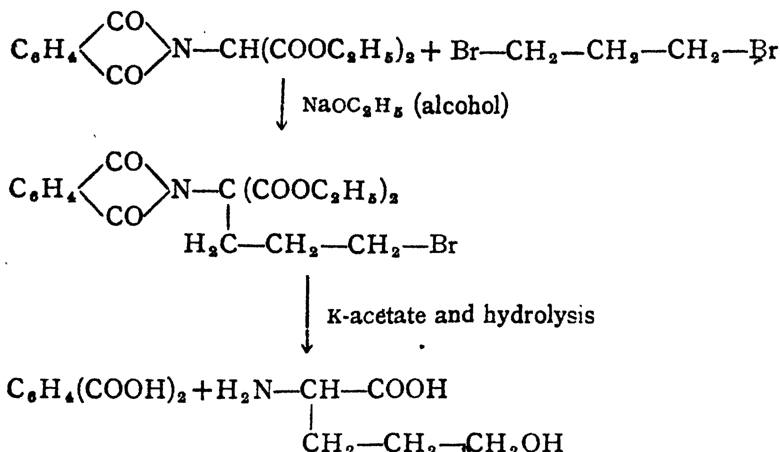


The compound A is then treated with Na in C_2H_5OH and subsequently with an alkyl halide to give B the corresponding alkylated derivative of A. The latter on boiling with hydro-chloric acid at high temperature, is hydrolysed to the amino acid.

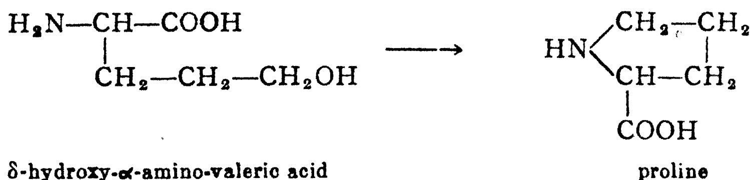


Better yields are obtained by saponifying the compound B with concentrated alkali and subsequently decarboxylating with concentrated HCl. The method can be extended to the synthesis of substituted

amino acids, by introducing suitable modifications. Thus δ-hydroxy-α-amino valeric acid can be obtained as follows:

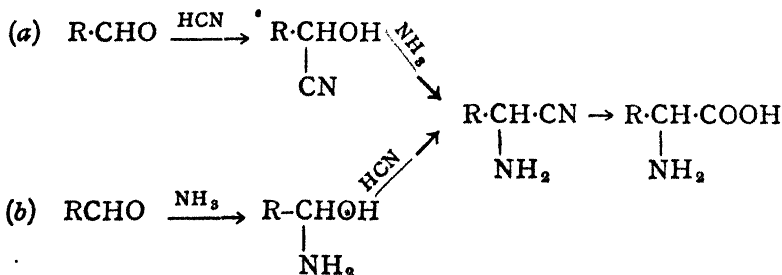


The latter acid on heating with or without hydrochloric acid, is converted into proline, one of the amino acids present in protein hydrolysate.

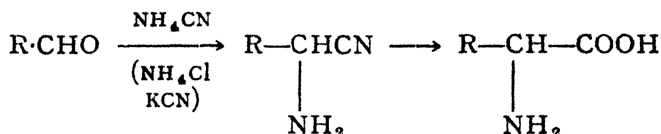


δ-hydroxy-α-amino-valeric acid proline

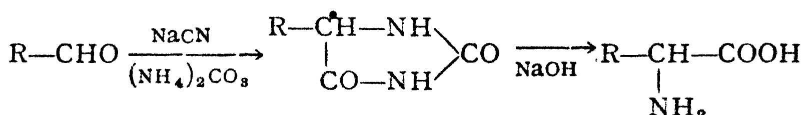
3. STRECKER'S SYNTHESIS :—This involves the condensation of aldehydes or ketones with ammonia and hydrocyanic acid; the nitriles of α-amino acids are formed which are hydrolysed to the acid.



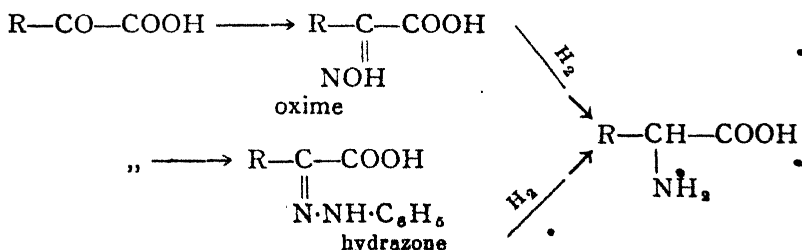
With ammonium cyanide, the nitrile is directly obtained and can be hydrolysed to the acid—



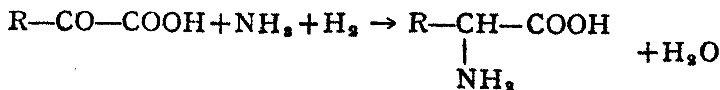
This synthesis is of wide application and can be extended to substituted aldehydes, Glycollic aldehyde is, thus, converted into β-hydroxy-α-amino propionic acid, $HOCH_2—CH(NH_2)—COOH$; it is the racemic form of serine from silk. In the Bucherer's modification of the method, the amino-nitrile is treated with $(NH_4)_2CO_3$ to form a hydantoin which is then hydrolyzed with alkali to give amino acid.



4. Reduction Methods ;— α-Amino acids can be prepared by the reduction of the oximes, nitriles or phenyl-hydrazones of ketonic acids :—



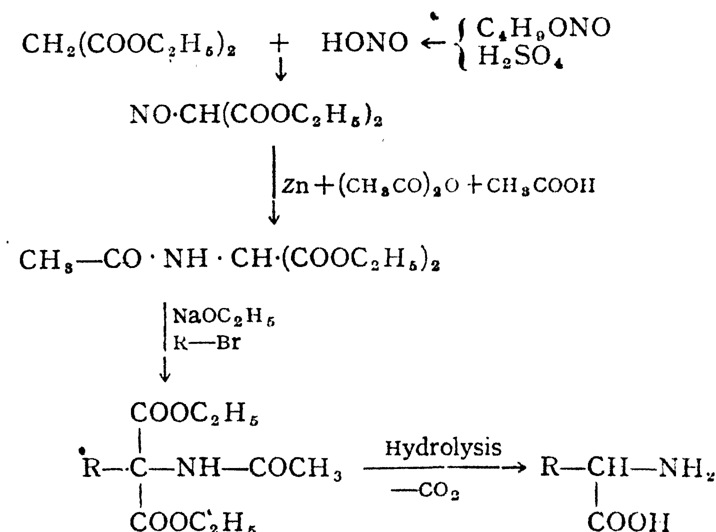
The reduction of the α-keto-acid to α-amino acid can be carried out by the action of nascent hydrogen (sodium amalgam etc.) or molecular hydrogen and Pd (catalytically) in presence of ammonia :



The imino acid $\underset{NH}{\overset{R—C—COOH}{\|}}$ is probably first formed which is then reduced to the amino acid. This is the Knoop's method.

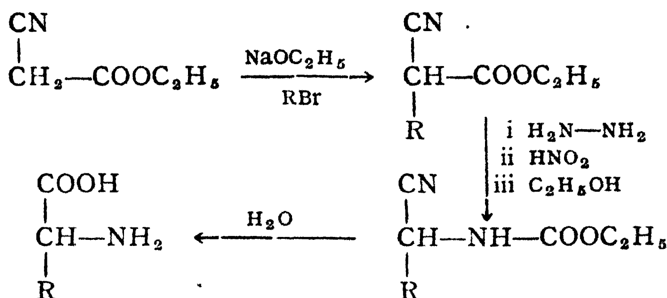
This reduction reaction appears to be an imitation of a reaction which takes place in the animal body. It constitutes an important link in the conversion of carbohydrates into α-amino acids and subsequently into proteins. (Carbohydrates → ketonic acids → amino acids → proteins).

5. α-Amino Acids From Malonic Ester :—Recently a method has been developed to synthesise α-amino acids, starting from malonic ester. Many amino acids of different types have been obtained by this method. The essential steps in the process are :



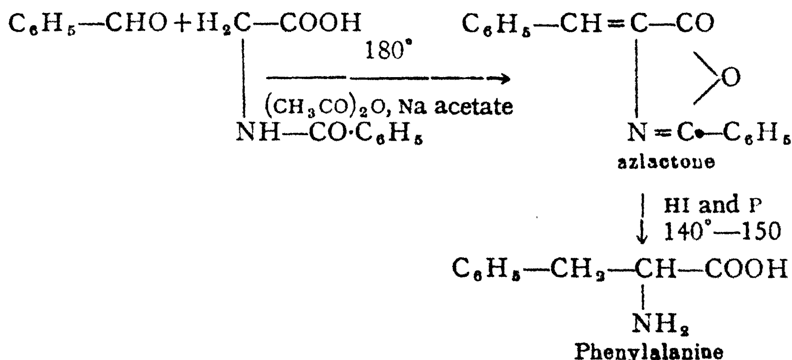
This method may be extended to Ar—CH_2X which will lead to the synthesis of α-amino acids containing an aryl residue.

Amino Acids From Cyanacetic Ester :—A method which starts from cyanacetic ester and which is based on the Curtius degradation has been increasingly employed for the synthesis of α-amino acids. The essential steps in the process are :



All the methods described above are applied to the preparation of aliphatic α-amino acids. They cannot be fruitfully used to synthesise the aromatic amino acids. A few methods have been developed, which are useful in the synthesis of the aromatic amino acids. They are (*i*) the azlactone method and (*ii*) the hydantoin method.

ERLENMEYER'S AZLACTONE METHOD: This method is applicable to the synthesis of α-amino acids containing aromatic residues and was developed by Erlenmeyer. It consists in heating an aromatic aldehyde with hippuric acid, which is benzoylglycine, in presence of fused Na-acetate and acetic anhydride, to form the azlactone (oxazolone); the latter is then converted by reduction and hydrolysis into the aromatic α-amino acid. The reactions involved can be formulated as follows:



Benzaldehyde may be replaced by other aromatic aldehydes. Tyrosine has been, thus, obtained by this method. *p*-Hydroxybenzaldehyde is heated with benzoyl-glycine in presence of acetic

anhydride and sodium acetate to give the corresponding azlactone. The latter, on heating with hydriodic acid and red phosphorus, gives tyrosine : *p*-hydroxy-phenylalanine.

$$HO-C_6H_4-CHO + H_2C(NH-CO\cdot C_6H_5)-COOH \longrightarrow HO-C_6H_4-CH{=}C-CO(-O-)\,N{=}C-C_6H_5$$

$$\xrightarrow{HI + P} HO-C_6H_4-CH_2-CH(NH_2)-COOH$$

tyrosine

In a recent modification the azlactone is hydrolysed with alkali; the unsaturated acid thus obtained is reduced with Na/Hg. Acid hydrolysis of the reduced product gives the α-amino acid.

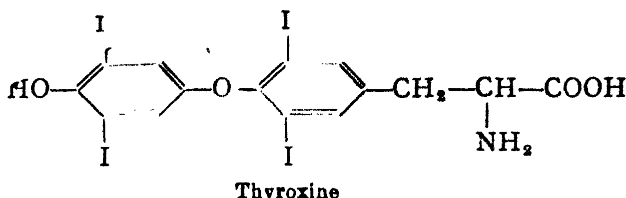
Tyrosine can be converted into thyroxine, the active principle of the thyroid gland, by condensing it with 2, 4, 6 tri-iodophenol and subsequent iodination.

$$HO-C_6H_2I_2-I + KO-C_6H_4-CH_2-CH(NH_2)-COOH$$

$$\downarrow$$

$$HO-C_6H_2I_2-O-C_6H_4-CH_2-CH(NH_2)-COOH$$

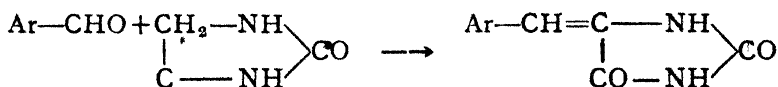
$$\downarrow \; I_2 \text{ in KI}$$



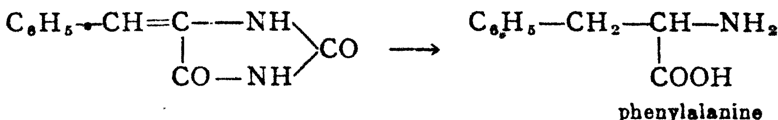
Thyroxine

The Erlenmeyer's method has been fruitfully used to synthesise such diverse type of amino acids as tryptophane, thyroxine, and phenylalanine.

HYDANTOIN METHOD : A useful variation of the above synthesis consists in the condensation of the aromatic aldehyde with hydantoin, in presence of acetic anhydride.

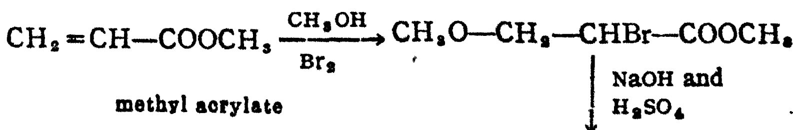


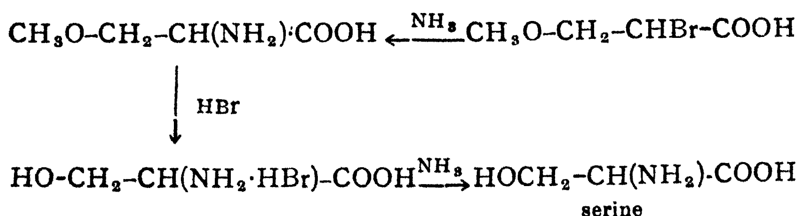
The condensation product, on tratment with ammonium sulphide at 60 to 100°, suffers reduction and hydrolysis to yield the α-amino acid.



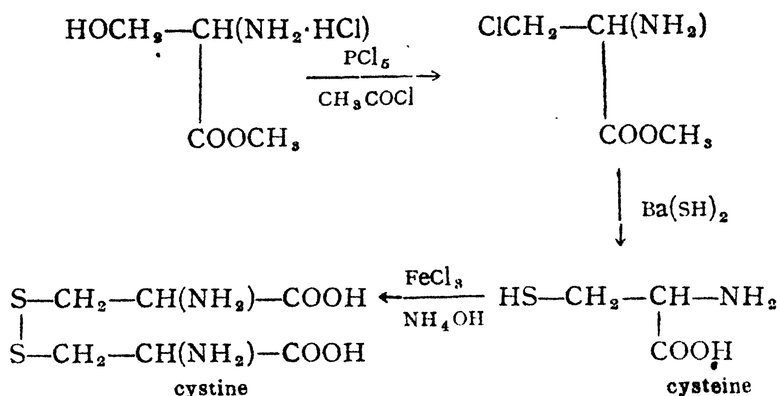
A recent improvement of the method involves the use of thiohydantoin, which is relatively cheaper and which gives better yields of the intermediate condensation product. This modified procedure is used in a recent synthesis of tyrosine.

Special Methods: The α-amino-β-hydroxy-acids cannot be satisfactorily obtained by the methods outlined above. They are better obtained through the α-halogenated-β-hydroxy acids by the action of ammonia. Serine, a typical α-amino β-hydroxy-acid is obtained as follows:

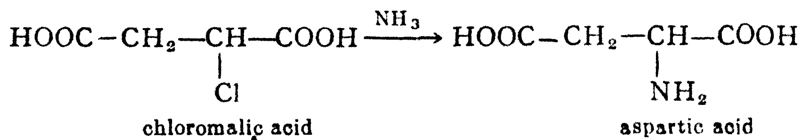




The sulphur containing amino-acids cysteine and cystine are readily obtained from serine, according to the following scheme:



The mono-amino-dicarboxylic acids are generally obtained from the corresponding mono-α-halogenated dibasic acids. Thus aspartic acid is obtained from chloromalic acid by the action of ammonia.



The di-amino-mono-carboxylic acids are rather difficult to prepare. A few of them have been obtained from the α-amino-dicarboxylic acids by the application of the Schmidt's reaction. The latter consists in the action of N_3H in $CHCl_3$ on the acid dissolved in con. H_2SO_4, and at the temperature range 45 to 50°.



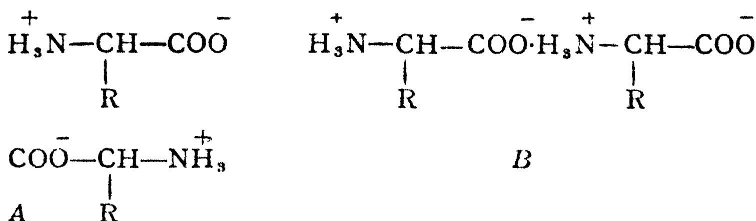
When applied to the α-amino di-carboxylic acids, it is found that the COOH group remote from the amino group is replaced by NH_2 group. The amino acids ornithine and lysine are obtained by this method in good yields.

General properties of α-amino acids: The amino acids are colourless, crystalline compounds soluble in water; cystine and tyrosine are insoluble. They are usually slightly soluble in alcohol and insoluble in ether (except proline and hydroxy-proline). All of them except glycine are optically active. The general structural formula for the α-amino acids is R—CH—(NH_2)—COOH. They thus, contain both the *carboxyl* and the *amino* groups. It has now been shown by their Raman spectra and other evidence, that in the solid form, and in aqueous solution they exist as '*dipoles*' *i. e.* in the form of an internal salt containing both positive and negative charges.

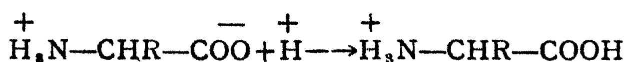


The net charge is, thus, zero and the ion is called '*dipolar ion*' or *Zwitter-ion.* These compounds thus are ionised, but they do not dissociate into ions like the salts. In their physical properties, they closely resemble salts; they are soluble in water but insoluble in ether. They are also non-volatile and infusible. On heating, they do not melt but are decomposed.

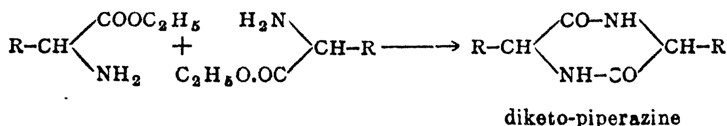
In the solid state, they are probably present as either A or B.



Under suitable conditions amino acids show the typical reactions of the carboxyl and the amino groups. In presence of hydrochloric acid, *i.e.*, $\overset{+}{H}$-ions, the negative charge is suppressed and a free carboxyl group is formed,

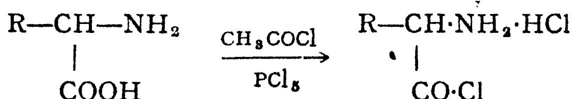


which can then be esterified. Thus, with absolute alcohol and hydrochloric acid, the amino acids are converted into their ethyl esters. The latter no loner resemble salts in their physical properties. They are volatile and can be distilled without decomposition under reduced pressure. On heating or allowing to stand for some time, the esters undergo condensation to give diketo-piperazines.



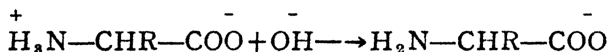
diketo-piperazine

The chlorides of the amino acids are obtained by the action of a large excess of phosphorus pentachloride in acetyl chloride.



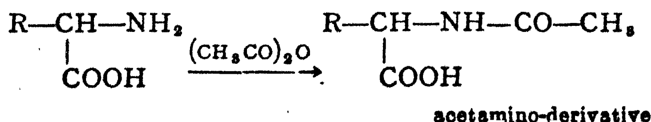
The acid chlorides are stable only in the form of their hydrochlorides. These chlorides have been used by Fischer in the synthesis of peptides (*q.v.*).

As acids, they form co-ordination complex salts specially with copper which are used in their separation and identification. In the presence of alkali, on the other hand, the amino acid molecule loses its positive charge :—



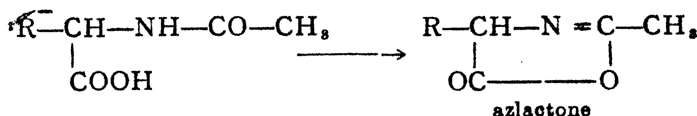
$$\overset{+}{H_3N}—CHR—CO\overset{-}{O}+\overset{-}{O}H \longrightarrow H_2N—CHR—CO\overset{-}{O}$$

i.e., a free amino group is generated. Hence, in alkaline conditions acylation *e. g.*, introduction of acetyl, benzoyl, arylsulphonyl group etc. takes place readily. Acetylation of the amino acids can take place to give the acetamino derivative or the azlactones. In alkaline solutions or with acetic acid in the cold, acetic anhydride forms the acetamino derivative :—

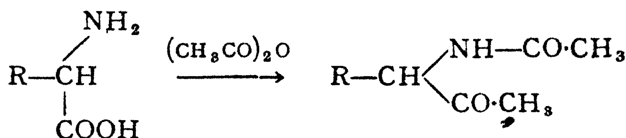


acetamino-derivative

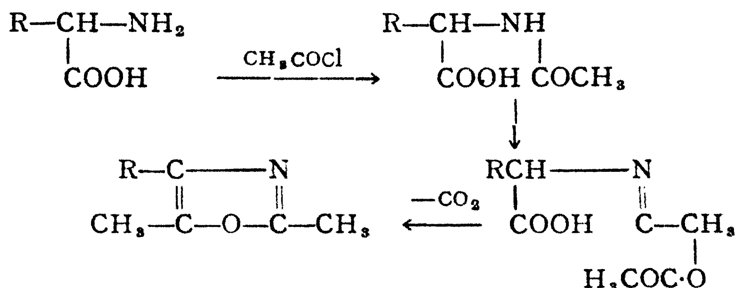
At high temperatures, with excess of the anhydride, the azlactone is obtained, the acetamino derivative suffering internal esterification :—



In presence of pyridine, acetic anhydride yields a methyl-acetamino-alkyl-ketone :—



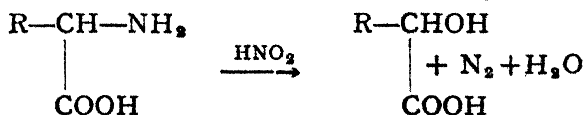
Oxazole derivatives are obtained when α-amino acids are heated with acetyl chloride in acetic acid :—



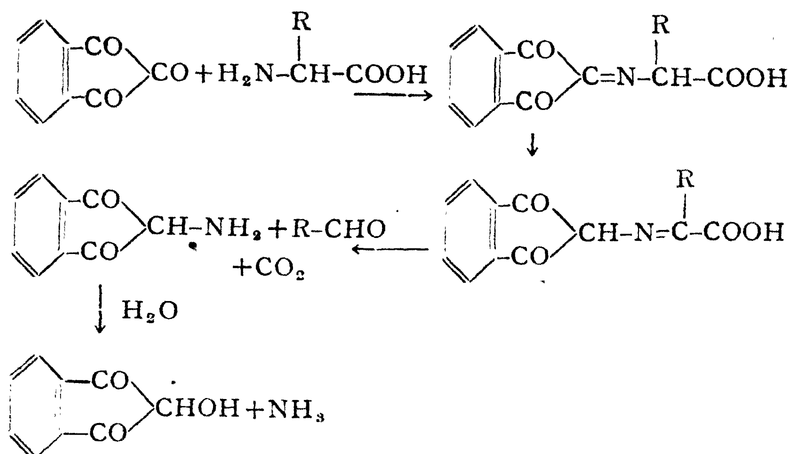
In all the above reactions, the enolisation of the acetamino group :—$NH—CO \rightarrow N=C—OH$ is involved.

The *N*-acyl derivatives of the amino acid, *e.g.*, benzoyl, 3-5-dinitro-benzoyl, and β-naphthalene-sulphonyl derivatives possess great analytical significance; they are strongly acidic compounds. They are crystalline compounds, sparingly soluble in water and nence, can be used for their separation and identification.

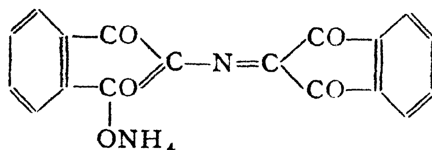
With nitrous acid (sodium nitrite in excess and acetic acid) the amino acid is converted into the corresponding hydroxy acid :—



This reaction is characteristic of a primary amino group. In the case *of the α–amino acids, it is quantitative and rapid with sodium* nitrite and acetic acid. The α–amino acids give a blue coloration with ninhydrin. This is a characteristic reaction. The reactions involved are :—



The blue colour is then associated with the formation of :



which is formed by the condensation of the ninhydrin, its reduction product, and NH_3.

Methods for estimation : There are several methods available for the quantitative estimation of the α-amino acids. The most commonly employed are :—

(1) Van Slykes method

(2) Sorenson's formol titration method

(3) Willstatter's method

(4) Linderstrom–Lang method

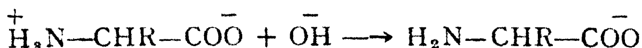
(5) Periodic oxidation method

(6) Ninhydrin method

Van Slykes method : The amino acid is treated with $NaNO_2$ in excess and acetic acid. N_2 *is evolved quantitatively which is* determined by a micro gasometric method. Half of the N_2 evolved corresponds to the nitrogen present in the acid. Most amino acids react normally. Glycine, Cystene, Serine all yield more than the theoretical amount of N_2. Lastly, no nitrogen is estimated which is present as NH or —N=groups; thus the nitrogen in histidine cannot be estimated: the terminal guanidine group $H_2N—C=NH—$ in arginine also cannot be computed by this method.

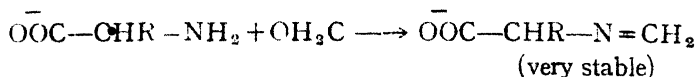
Sorenson's method : The aqueous solution of the amino acid is treated with excess of a carefully neutralised formaldehyde solution and titrated with a standard alkali using phenolphthalin as the indicator. The changes involved are :

(a) on the addition of alkali, the dipolar ion $\overset{+}{N}H_3\text{-CHR-CO}\overset{-}{O}$ is converted into $H_2N—CHR—CO\overset{-}{O}$:—



$$\overset{+}{H_3}N—CHR—CO\overset{-}{O} + \overset{-}{O}H \longrightarrow H_2N—CHR—CO\overset{-}{O}$$

(*b*) the free NH_2 group then condenses with formaldehyde to form the stable ion :—



$$\overset{-}{O}OC—CHR—NH_2 + OH_2C \longrightarrow \overset{-}{O}OC—CHR—N=CH_2$$

(very stable)

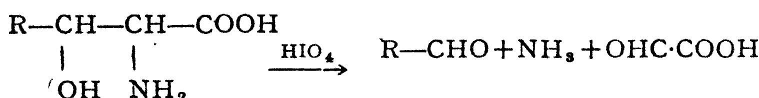
Thus, the reaction consists in the neutralisation of the positive charge as formulated above; it is therefore the amino group that is actually being estimated. According to Hawrowitz, formaldehyde condenses with the NH_2 group to form mono or dimethylol compounds of the type : $NH—CH_2OH$ (mono) and $N(CH_2OH)_2$ (di). The formation of such compounds greatly reduces the basicity of the NH_2 group, and thus the carboxyl group is freely and quantitatively titrated by the alkali.

In the *WILLSTATTER'S METHOD* the titration is carried out in ethanol (97–100%). The titration in ethanol is rendered possible by the shift of the pK value of phenolphthalein used as the indicator; while the colour change in aqueous solution occurs at pH 9, it takes place in ethanolic solution near about pH 12; at this high pH value, the positively charged $\overset{+}{N}H_3$ groups are converted into uncharged NH_2 groups, which cannot neutralise the anionic COO group.

LINDERSTROM-LANG METHOD:—The principle of this method is entirely different, as it estimates the carboxyl group. The amino-acid in aqueous acetone solution is titrated with alcoholic hydrochloric acid, using naphthyl—red as the indicator. The neutralisation in this case involves the elimination of the negative charge.

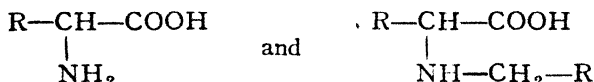


PERIODATE METHOD: The β-hydroxy-α amino acids, serine and threonine are estimated by a method based on oxidation by periodic acid.



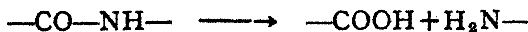
In the case of serine, the aldehyde formed is CH_2O, while threonine yields acetaldehyde. The amounts of each of the two acids are then determined by estimating the aldehydes.

The Ninhydrin method: It is specially used for the amino acids which give abnormal values with the Van Slyke's method. In this method, the CO_2 evolved between pH 1–5, at the temperature of the boiling water is estimated. The reaction is quantitative with acids:



Polypeptides

The results of hydrolysis of the complex natural proteins, indicate that there are twenty-three different α-amino acids which lie at the basis of their complex structure. The proteins originally contain relatively few free *amino* and free carboxyl groups; their number however goes on increasing, as the hydrolysis progresses. Further an equal amount of NH_2 and COOH groups are liberated. The ready separation of these units by hydrolysis, suggested to Fischer and Hofmeister that the amino acids are present in the protein molecule as linked together by the CO—NH—linking, called the peptide linking. Such a grouping which is essentially an acid amide type of linking is readily hydrolysable to give simultaneously a free amino and a free carboxyl group.

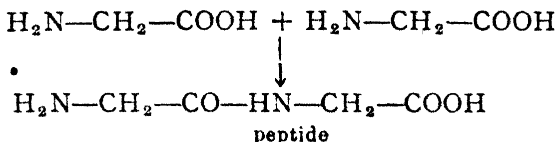


$$-CO-NH- \longrightarrow -COOH + H_2N-$$

This would satisfactorily account for the results of hydro ysis of the proteins.

Fischer, guided by the above reasoning based on analytical results, defined proteins as naturally occurring substances of high molecular weight, which are largely composed of α-amino acid residues united together, by an amide type of linking, to form a straight chain. He next proceeded to verify his views and conclusions by resorting to the synthetical method. He developed successful methods, by which the molecules of α-amino acids could be *successively* linked on to one another in a series of amide formation, each intermediate compound being isolated and identified. Thus, Fischer initiated and developed the study of *polypeptides* or synthetic proteins. A polypeptide is a molecule built up of many α-amino acid residues linked by the CO–NH–group. They are intermediate between the simple α-amino acids and the complex proteins.

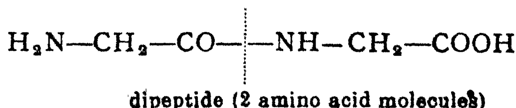
The formation of the peptide chain by condensation of the α amino acids, takes place as follows :



$$H_2N-CH_2-COOH + H_2N-CH_2-COOH \downarrow H_2N-CH_2-CO-HN-CH_2-COOH$$

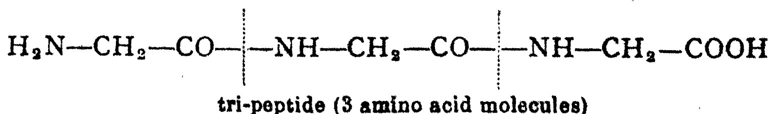
peptide

The products so obtained are termed di-, tri-, tetra-peptides etc. depending on the number of amino acid residues contained in the molecule. Thus, we have :—



$$H_2N-CH_2-CO-\vdots-NH-CH_2-COOH$$

dipeptide (2 amino acid molecules)



$$H_2N-CH_2-CO-\vdots-NH-CH_2-CO-\vdots-NH-CH_2-COOH$$

tri-peptide (3 amino acid molecules)

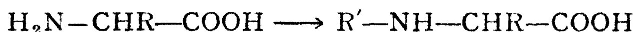
The tri-peptide can react further at each end of the molecule, with more and more amino acids forming peptides containing several amino acids.

The compounds containing many peptide linkings are then called polypeptides. In the peptide formation, several ∝-amino acids can take part in the peptide chain formation; further the relative order of combination may also vary and thus the number of possible combinations is very great. Thus, on the basis of this hypothesis, Fischer could explain satisfactorily the existence of a large number of different natural proteins, built up from only twenty-three different ∝-amino acids.

Synthesis of Polypeptides

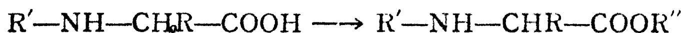
Fischer has developed a series of methods for synthesising polypeptides. The ∝-amino acids required are synthesised by one of the methods outlined. The peptides are the condensation products of two or more ∝-amino acids; and as the latter exist in the form of the dipolar ion $\overset{+}{H_3N}—CHR—C\overline{OO}$, *i. e.*, an internal salt, special methods have to be employed for an effective condensation. The underlying principles of an effective peptide synthesis are :—

(*i*) The amino group of a molecule of an ∝-amino acid is protected by introduction of a suitable substituent *R′* into the NH_2 group. (The basic properties of the amino acid are reduced to a minimum). A number of groups like C_6H_5CO, $CO{\cdot}OC_2H_5$ and $C_6H_5—CH_2—O{\cdot}CO$—have been introduced with practical advantage.

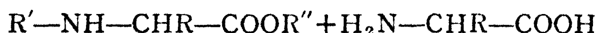


$$H_2N-CHR—COOH \longrightarrow R'—NH—CHR—COOH$$

(*ii*) The carboxyl group is converted into a suitable derivative —*COOR″*, which is very reactive and would condense readily with the free amino group of another amino acid molecule :—



$$R'—NH—CHR—COOH \longrightarrow R'—NH—CHR—COOR''$$



$$R'—NH—CHR—COOR''+H_2N—CHR—COOH$$

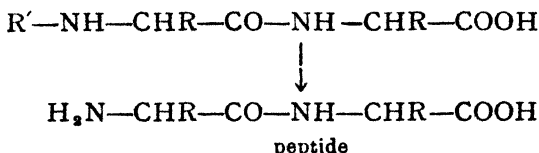




$$R'—NH—CHR—CO—HN—CHR—COOH$$

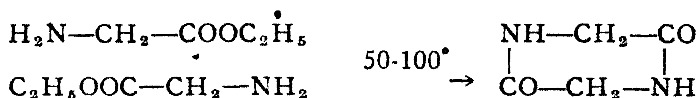
Esterification and chloride formation of the carboxyl group have been utilised by Fischer and others.

(*iii*) Lastly, the protecting group is removed by hydrolysis or by other simple methods of elimination under conditions which do not attack the peptide linking, to give the peptide:

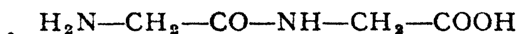


We shall now discuss the different synthetic methods developed by Fischer and others:—

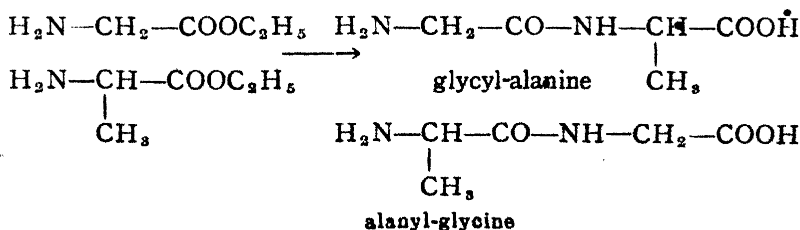
1st Method:—Fischer obtained the first peptide by the condensation of two molecules of an amino ester, with the elimination of a molecule of alcohol; the main product of the reaction is the di-keto-piperazine.



On hydrolysis with HCl, the diketo-piperazine yields the dipeptide :

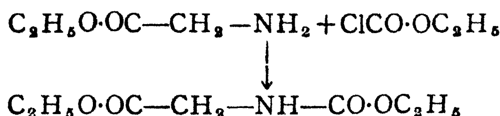


This method has some practical disadvantages: (*i*) it is limited to the preparation of di-peptides of amino acids that form anhydrides, (*ii*) when applied to a mixture of two different amino esters mixed diketo-piperazines would be formed which on acid hydrolysis, would give a mixture of two different peptides. Thus glycine and alanine would give both glycyl-alanine and alanyl-glycine.



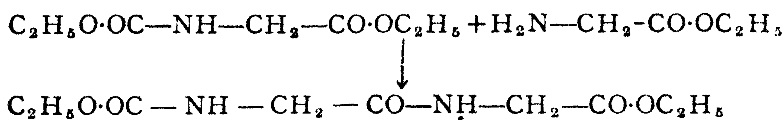
Such a mixture is difficult to separate and hence, the method possesses very little synthetic value. Fischer overcame this difficulty in his second method.

2nd Method :—In this method a *chosen* amino group is free to condense with the carboxyl group of another molecule of amino acid ester; the amino group of the latter is protected by introduction of a suitable group like carbo-ethoxy, $CO{\cdot}OC_2H_5$. The different steps involved are :—



$$C_2H_5O{\cdot}OC{-}CH_2{-}NH_2 + ClCO{\cdot}OC_2H_5 \downarrow C_2H_5O{\cdot}OC{-}CH_2{-}NH{-}CO{\cdot}OC_2H_5$$

The linking is then achieved by condensing the above molecule with another molecule of amino ester (heating for thirty-six hours).

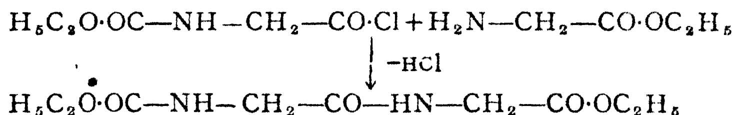


$$C_2H_5O{\cdot}OC{-}NH{-}CH_2{-}CO{\cdot}OC_2H_5 + H_2N{-}CH_2{-}CO{\cdot}OC_2H_5 \downarrow C_2H_5O{\cdot}OC{-}NH{-}CH_2{-}CO{-}NH{-}CH_2{-}CO{\cdot}OC_2H_5$$

The carbo-ethoxy group is then eliminated to give the dipeptide.

This synthesis gave a peptide of *known* and *pre-determined* constitution. But Fischer found it difficult to remove the carbo-ethoxy group and hence, the yields of the end- product were poor. On prolonged hydrolysis with alkali, the above ester gave a di-basic acid and not the dipeptide as the main product.

3rd Method :—This method depends on the condensation of an acid chloride of carbo-ethoxy derivative or an amino acid with the amino group of an amino ester :—



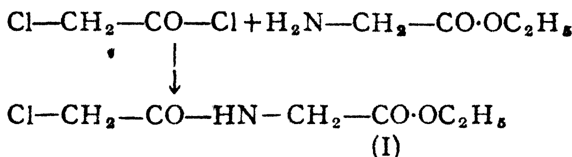
$$H_5C_2O{\cdot}OC{-}NH{-}CH_2{-}CO{\cdot}Cl + H_2N{-}CH_2{-}CO{\cdot}OC_2H_5 \xrightarrow{-HCl} H_5C_2O{\cdot}OC{-}NH{-}CH_2{-}CO{-}HN{-}CH_2{-}CO{\cdot}OC_2H_5$$

The yields of the peptide derivate are greatly improved. The acid chloride is obtained by the action of thionyl chloride on the amino acid. However, as the carbo-ethoxy group cannot be readily removed without attacking the peptide linkage at the same time, the foregoing methods cannot be used for the building up of higher poly-peptides.

Fischer then devised and developed the fourth method by which polypeptides of any desired constitution and length could be secured.

4th Method :—The essential steps of this method are :—

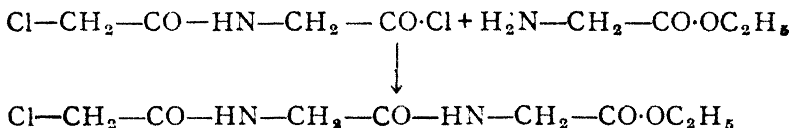
(*i*) The primary condensation between an amino ester and chloro-acetyl-chloride or bromo acetyl-bromide is effected to give the compound (I)—



$$Cl—CH_2—CO—Cl + H_2N—CH_2—CO·OC_2H_5 \downarrow Cl—CH_2—CO—HN—CH_2—CO·OC_2H_5 \text{ (I)}$$

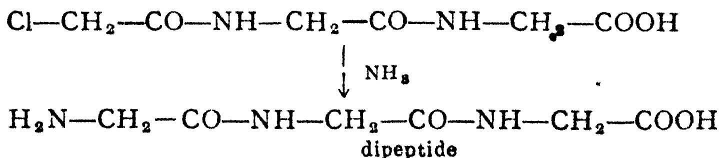
It is obvious that the NH_2 group is incidentally protected; further the additional advantage of this technique is that this group affording the protection has not to be removed.

(ii) The compound (I) is carefully hydrolysed with dilute alkali and then converted into the chloride with $SOCl_2$. The chloride is made to interact with another molecule of the α-amino acid or a peptide, thus lengthening the chain at the COOH end :—



$$Cl—CH_2—CO—HN—CH_2—CO·Cl + H_2N—CH_2—CO·OC_2H_5 \downarrow Cl—CH_2—CO—HN—CH_2—CO—HN—CH_2—CO·OC_2H_5$$

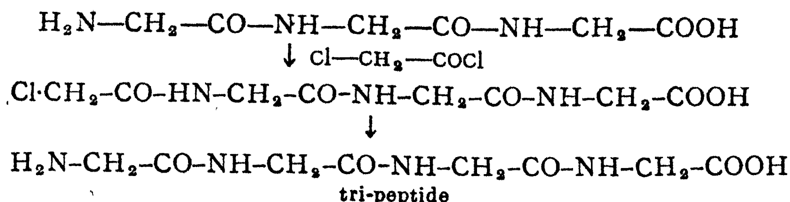
The $CO·OC_2H_5$ group is then hydrolysed to $COOH$ and the halogen atom is replaced by NH_2 group by the action of ammonia to give peptide. Amination before hydrolysis would lead to the formation of amide, $CO·OC_2H_5 \rightarrow CO—NH_2$ which is very difficult to separate.



$$Cl—CH_2—CO—NH—CH_2—CO—NH—CH_2—COOH \xrightarrow{NH_3} H_2N—CH_2—CO—NH—CH_2—CO—NH—CH_2—COOH$$

dipeptide

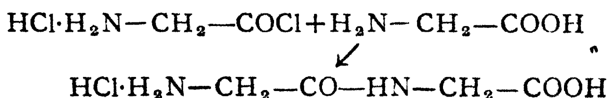
The lengthening of the peptide chain may be effected at the amino end; this involves the condensation of the peptide with chloro-acetyl-chloride and subsequent reaction with ammonia to replace the halogen atom—



5th Method :—A better and more elastic method was finally evolved by Fischer. It consists in the condensation of the acid chlorides of the hydrochlorides with amino acids or esters.

The acid chlorides alone are difficult to prepare. The hydrochlorides of the acid chlorides, on the other hand, are readily obtainable by the action of phosphorus pentachloride in acetyl chloride on the amino acids; special precautions are necessary to protect them from atmospheric moisture.

The hydrochloride of the acid chloride may be condensed with a peptide, instead of an amino acid or ester, to give the higher peptide.



By the applications of the foregoing methods with many ingenious variations, Fischer (1907) could build up the largest molecule of known structure ever produced by a synthetic method. It is *octa-deca-peptide*, (*l*-leucyl-tri-glycyl-*l*-leucyl-tri-glycyl-*l*-leucyl-octa-glycyl-glycine), a peptide with *eighteen* amino acid molecules and a molecular weight of 1213. Nine years later, Abderhalden and Fodor (1916) achieved the synthesis of *nona-deca-peptide*, with a molecular weight of 1326.

However, this method breaks down in the case of syntheses involving hydroxy and amino aromatic acids. Phosphorus pentachloride has been known to react with them in a complicated way and the expected halogen derivatives are not formed.

We will now discuss the methods developed at the same time, by Curtius. They are based upon different principles; in one of

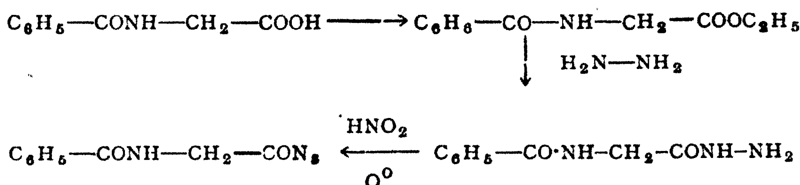
the methods, the benzoylated amino acid azide is condensed with a molecule of amino acid ester, in alkaline conditions to give the peptide.

(*a*) preparation of the benzoylated acid azide.



$$C_6H_5COCl + H_2N\text{-}CH_2COOH \rightarrow C_6H_5\text{-}CONH\text{-}CH_2\text{-}COOH$$

The benzoylated acid is then converted into the azide as follows



$$C_6H_5\text{—}CONH\text{—}CH_2\text{—}COOH \longrightarrow C_6H_5\text{—}CO\text{—}NH\text{—}CH_2\text{—}COOC_2H_5 \xrightarrow{H_2N\text{—}NH_2}$$

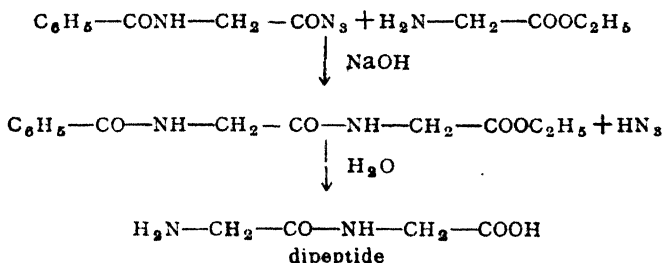
$$C_6H_5\text{—}CONH\text{—}CH_2\text{—}CON_3 \xleftarrow[0^\circ]{HNO_2} C_6H_5\text{—}CO\cdot NH\text{-}CH_2\text{-}CONH\text{-}NH_2$$

The azide may be obtained from the corresponding acid chloride by the action of NaN_3 in acetone solution.



$$R\text{—}CO\cdot Cl + NaN_3 \longrightarrow \underset{\text{acid azide}}{R\text{—}CO\cdot N_3} + NaCl$$

(*b*) *The peptide formation :* The azide is then condensed with an amino acid ester according to the following equation.



$$C_6H_5\text{—}CONH\text{—}CH_2-CON_3 + H_2N\text{—}CH_2\text{—}COOC_2H_5 \xrightarrow{NaOH}$$

$$C_6H_5\text{—}CO\text{—}NH\text{—}CH_2-CO\text{—}NH\text{—}CH_2\text{—}COOC_2H_5 + HN_3 \xrightarrow{H_2O}$$

$$\underset{\text{dipeptide}}{H_2N\text{—}CH_2\text{—}CO\text{—}NH\text{—}CH_2\text{—}COOH}$$

However the yields are poor, because the difficulty lies in removing the benzoyl group without at the same time breaking down the peptide linking. This difficulty is partly overcome by Curtius in his second method. In the latter method, he uses the tosyl group (pCH$_3-C_6H_4-SO_2-$) for protecting the amino group. It is subsequently removed by warming with HI at 50 to 60° or with HBr in glacial acetic acid in presence of phenol.

The essential steps involved are :

$$CH_3-C_6H_4-SO_2-Cl + H_2N-CH_2-COOC_2H_5$$

$$\downarrow \text{pyridine}$$

$$CH_3-C_6H_4-SO_2-NH-CH_2-COOC_2H_5$$

$$\downarrow H_2N-NH_2,\ HNO_2$$

$$CH_3-C_6H_4-SO_2-NH-CH_2-CON_3$$

$$\downarrow H_2N-CH_2-COOC_2H_5$$

$$CH_3-C_6H_4-SO_2-NH-CH_2-CO-NH\text{-}CH_2\text{-}COOC_2H_5$$

$$\downarrow \text{HI } (50\text{—}60^\circ)$$

$$\underset{\text{thiocresol}}{CH_3-C_6H_4-SH} + \underset{\text{dipeptide}}{H_2N-CH_2-CO-NH-CH_2-COOH}$$

All the methods discussed above are not applicable to the synthesis of polypeptides containing aromatic residues. This difficulty has been overcome by Bergmann in his azlactone method.

BERGMANN'S AZLACTONE METHOD :—This is a special method devised by Bergman to introduce aromatic acid residues into an amino acid. Thus peptides containing amino aromatic acid molecules can be obtained by this method. The preparation of *d*-tyrosyl-*d*-arginine is as follows :—

The acetyl derivative of *p*-hydroxy-benzaldehyde is heated with acetyl-glycine in presence of acetic anhydride and sodium acetate to give the azlactone.

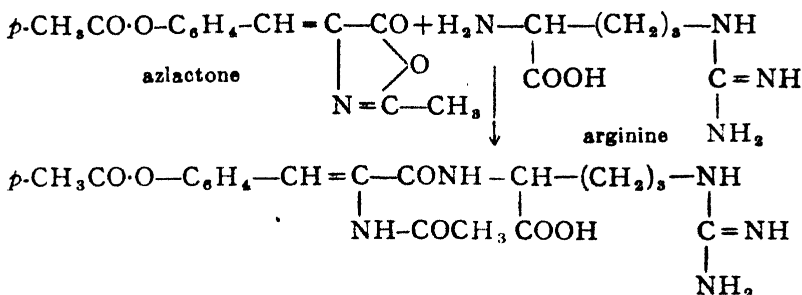
$$CH_3\cdot CO\cdot O-C_6H_4-CHO + H_2C(NH-COCH_3)-COOH$$

$$\downarrow (CH_3CO)_2O,\ \text{Na-acetate}$$

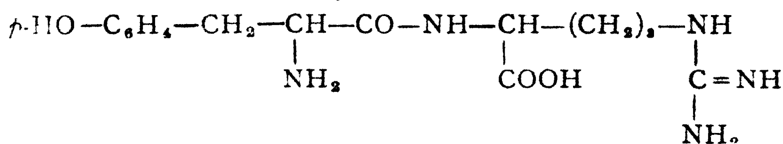
$$CH_3CO\cdot O-C_6H_4-CH=C-CO \quad (\text{ring: } C-CO-O-C(CH_3)=N-C)$$

$$N=C-CH_3$$

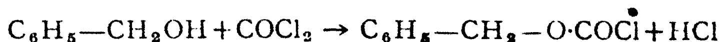
The azlactone is then made to interact with *d*-arginine. The amino group in α-position to the *COOH* group reacts with the lactone group :—



The latter on catalytic reduction with Pd and H_2 and subsequent elimination of the acetyl groups, by hydrolysis with HCl, gives the *di*-peptide *d*-tyrosyl-*d*-arginine :

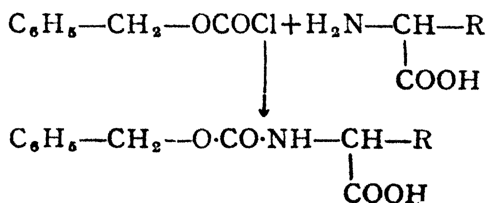


Lastly, we have the Bergmann's second method; this is more general and is of wider application than the foregoing one. It can be used to synthesise polypeptides from different α-amino acids. It is based on the fundamental principles of synthesis mentioned earlier. The amino group of the amino acid is protected by the carbo-benzyloxy-group $C_6H_5—CH_2·O·CO—$. This group is introduced into the amino acid by the reagent $C_6H_5—CH_2—O·COCl$. The latter is obtained by the action of $COCl_2$ in toluene, on benzyl alcohol.

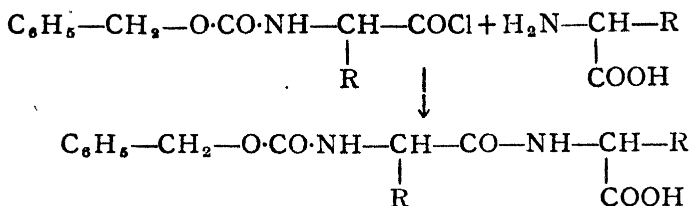


$$C_6H_5—CH_2OH + COCl_2 \rightarrow C_6H_5—CH_2—O·COCl + HCl$$

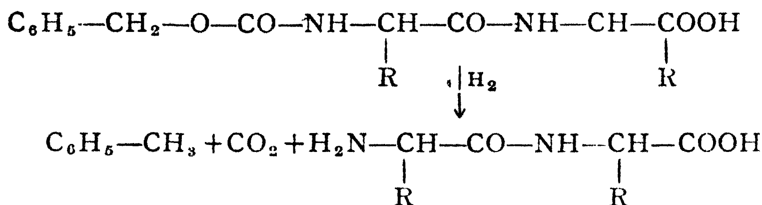
(*i*) It reacts with the amino group in alkaline solution to give the corresponding carbo-benzyloxy derivative.



(*ii*) The acid chloride of the above acid is made to interact with a moelcule of another or same amino acid, to give the peptide.



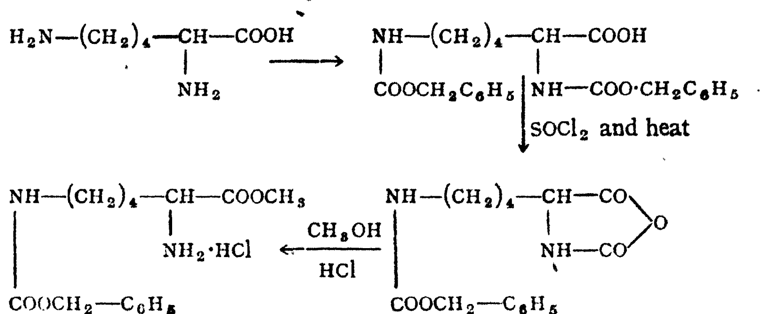
(*iii*) The protecting group is then removed by hydrogenolysis *i. e.* with hydrogen in presence of Pd or Pt at room temperature.



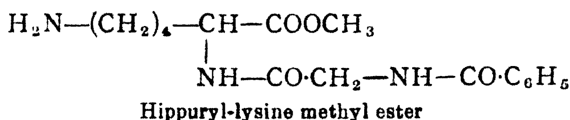
Thus the possibility of the hydrolytic cleavage of the peptide linking is completely eliminated and the yields of the peptide are thus increased. Thus, this protecting group has distinct advantages over the other methods of protecting the amino groups. The Bergmann's technique has another advantage in that the optically active amino acids used in the peptide synthesis are not at all racemised; thus it is rendered possible, to prepare peptides which are optically active and retain the stereo-isomeric configuration of the natural products. However catalytic hydrogenation cannot be used in the preparation of peptides which contain sulphur; because the latter poisons the catalyst and the hydrogenolysis does not proceed at all. This difficulty has been overcome by reducing the carbo-benzyloxy group with phosphonium iodide or with Na in liquid ammonia.

Lastly, by further slight modifications, the Bergmann's method can be used to synthesise peptides from (*i*) di-amino-mono-carboxylic acids and (*ii*) mono amino-di-carboxylic acids. In this way, a peptide containing lysine and another containing aspartyl or glutamyl residues have been obtained.

(a) Synthesis of hippuryl-l-lysine methylester.

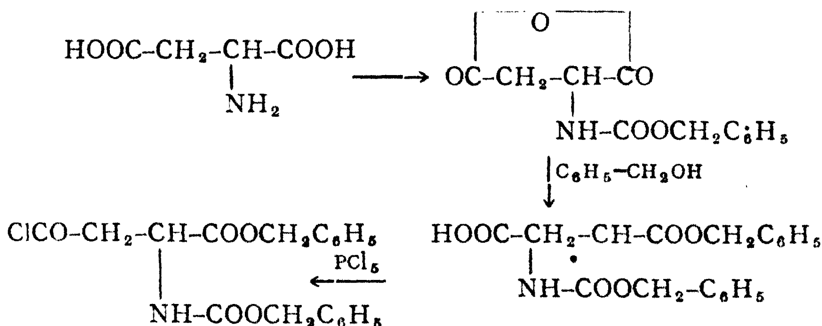


The free ester is then obtained by the action of K_2CO_3 and it is subsequently condensed with hippuryl chloride to give carbo-benzyl-oxy derivative of hippuryl-lysine peptide which on hydrogenolysis gives the peptide:



Hippuryl-lysine methyl ester

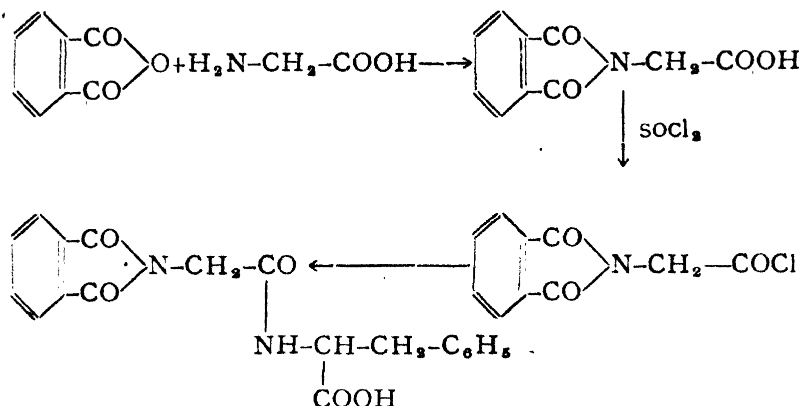
(b) Synthesis of aspartyl peptides :—



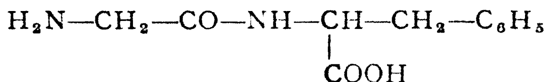
The chloride may be condensed with an amino acid to give the peptide derivative which is then converted into the free dipeptide by reduction with H_2 in presence of Pd at ordinary temperature.

SHEEHAN PHTHALYL SYNTHESIS OF PEPTIDES :—In this method, the amino group is protected by the phthalyl residue; the

phthalyl-peptide derivative formed, has excellent crystallising properties.



The latter on heating with hydrazine and subsequent treatment with HCl gives the dipeptide :



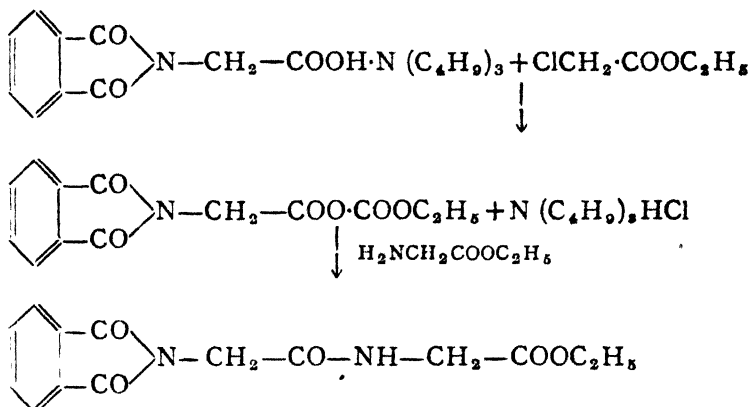
$$H_2N—CH_2—CO—NH—\underset{\displaystyle COOH}{\underset{|}{CH}}—CH_2—C_6H_5$$

The cleavage of the phthalyl dipeptide proceeds more readily than the hydrogenolysis of the carbobenzoxy group in the Bergmann's method.

Recently a new technique based on the formation of mixed anhydride has been developed by Wieland and others. The mixed anhydride is of the carbonic-carboxylic anhydride type. It seems to be the best method, as far as the yields and the prevention of racemisation are concerned. The following synthesis of glycyl-glycine will serve as an example.

Ethyl chloroformate is added to a cooled chloroform or tetrahydro furan solution of phthaloyl glycine and *n*-tri-butylamine; after allowing to stand for 10 mins. at 10°, the mixture is treated with a chloroform solution of ethylglycinate and *n*-tri-butylamine.

CO_2 is evolved and the phthaloyl-glycyl-glycine ethyl ester is precipitated.



On careful hydrolysis with—$H_2N—NH_2$ the peptide glycyl-glycine is obtained in good yields. The method is simple and versatile and is applicable to the synthesis of many peptides.

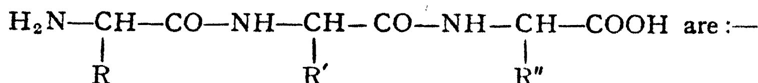
Natural polypeptides :—Recently many polypeptides have been isolated from fungi and from micro-organisms. Two of such compounds are gramicidin and tyrocidine isolated from soil micro-organisms. On hydrolysis, gramicidin gives ethanolamine, glycine *l*-alanine, *l*-tryptophane, *l*-and *d*-valine and *d*-leucine. Synge wa- able to show the arrangement of the amino acids in the peptide. He separated, by means of the two dimensional chromatographic method, *di*-and *tri*-peptides from gramicidin. The latter were then hydrolysed to amino acids. On the basis of these results, he has proposed the following peptide chain structure for gramicidin: Valine-ornithines leucine-phenylalanine-proline.

Tyrocidine also yields a number of amino acids on hydrolysis. They have been investigated as possible bactericidal agents; but they have not extensive applications owing to their toxicity.

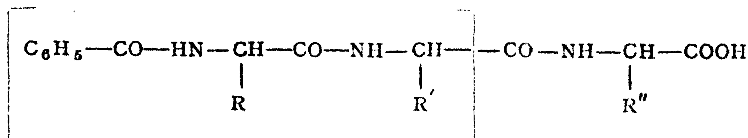
Glutathione—a sulphur containing compound and which takes part in biological oxidations, is a tripeptide. The sulphhydryl group from the peptide G-SH is oxidised to the disulphide from G-S-S-G.

Lastly, phalloidine from the death cap fungus and lycomarasmine from the cultures of the phytopathogenic fungus have been shown to be peptides in structure.

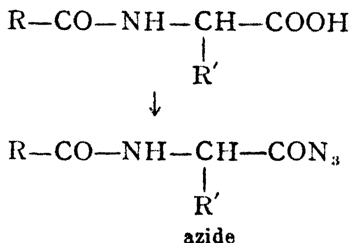
Also many peptides have been isolated from the product of enzymatic hydrolysis of the natural proteins. The nature of their constituent α-amino-acid can be established by a study of the products of hydrolysis. However, the question of the order of the amino acids in the peptide chain cannot be settled by the simple hydrolytic method, nor by a synthetic method. A degradation method has been evolved by Bergmann and Servas, which splits off the chain bit by bit at the carboxylic end; in principle, it is a combination of Bergmann's carbo-benzyloxy hydrogenation method and Curtius' azide degradation method. The essential steps in the degradation of a peptide:



(*a*) The free amino group is benzoylated to give the benzoyl peptide :—

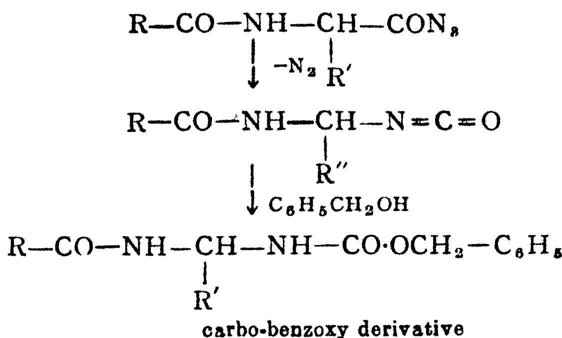


(*b*) The benzoyl peptide is then converted into an azide *via* the ester and the hydrazide.



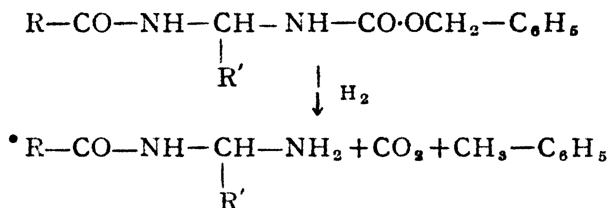
(*c*) The azide is warmed with benzyl alcohol when nitrogen is lost and it rearranges itself to an iso-cyanate (Curtius' rearrangement):

the isocyanate then adds on a molecule of benzyl alcohol to yield the carbo-benzoxy derivative :

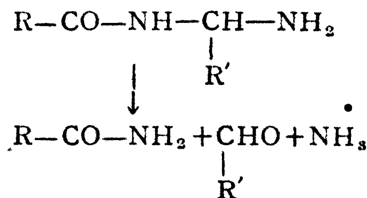


carbo-benzoxy derivative

(*d*) The carbo-benzyloxy derivative is then catalytically hydrogenated; the product is a benzoyl derivative of a di-amine (a fresh NH_2 group is now generated) :—



(*e*) The diamine derivative, on treatment with water, is hydrolysed to an aldehyde and the amide of a peptide with one less amino-acid residue :—



The aldehyde is then identified by standard methods and this establishes the constitution of the terminal amino-acid residue. The formation of the aldehyde takes place under conditions which do not involve the splitting of the peptide linkage. The process is then repeated and the nature of the terminal α-amino-acid residues, is

established one by one. Thus, the different α-amino-acid residues can be identified and then order in the peptide chain can be readily determined.

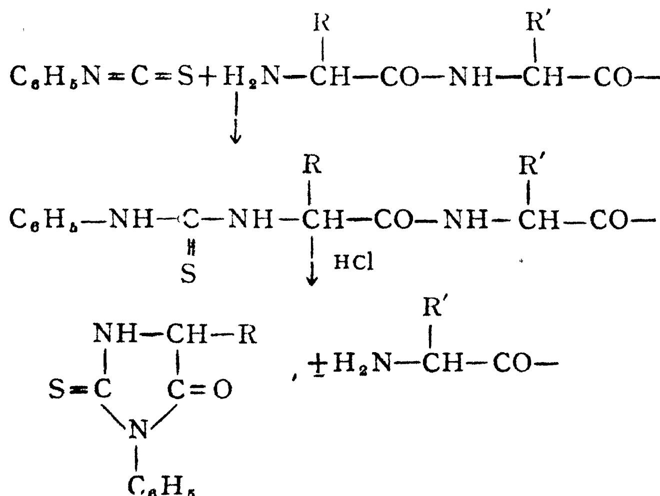
Other chemical methods have been developed to determine the 'order' of the amino acid residues in the polypeptide of the protein. In these methods, the terminal amino acid residue is removed one by one. The degradation of the chain may be introduced either at the amino-end or at the carboxyl-end.

The N-terminal (end) degradation : This is accomplished by two methods : (a) Sanger's method and (b) Edman's method.

Sanger's method : The reagent is D. N. F. (dinitro-fluro-benzene). The protein in aqueous alcoholic solution is reacted with D. N. F. in presence of $NaHCO_3$. The yellow D. N. Protein derivative is then hydrolysed with acid ; the peptide bonds are attacked but not the D. N.-nitrogen bond. The D.N. amino acid is extracted from the hydrolysate, separated and identified by partition chromatography using silicagel.

Edman's method : The reagent is $C_6H_5N=C=S$

The protein is made to react with the reagent in pyridine-water at pH 9. The phenyl-thio-carbonyl derivative is cleaved by saturated HCl acid in nitromethane or glacial acetic acid. The reactions involved are :—



Thiohydantoin thus formed on hydrolysis with Ba $(OH)_2$ gives the terminal H_2N-CH-COOH acid. The process is repeated with

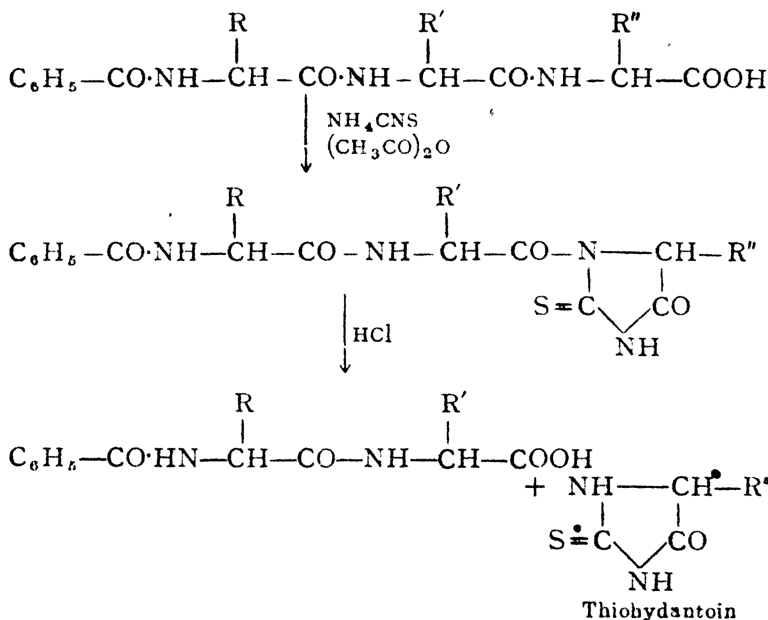
R

the degraded protein to split off one more N-terminal acid.

Carboxyl-end degradation methods: Several methods are known but the two commonly used are:

(1) Shlack-Kumpf's thiohydantoin method (2) Reduction methods.

Thiohydantoin method: The protein is first benzoylated to protect the end NH_2-group. The protein molecule is then heated with NH_4CNS and $(CH_3CO)_2O$; a thiohydantoin is formed out of the carboxyl-end amino acid. The reactions involved are:



Thiohydantoin

The latter on hydrolysis with Ba $(OH)_2$ gives the terminal amino acid H_2N—CH—COOH. The method is then repeated with
R″

the truncated protein and the amino acids from the carboxylic end can be removed step by step.

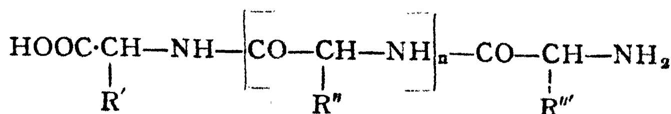
Reduction method : In this method, the free terminal CO_2H group is reduced to CH_2OH. The reduced protein is hydrolysed and the amino alcohol formed, separated and identified. The reduction is effected with $LiAlH_4$, and the separation of the hydrolysate is carried out chromatographically with silica gel.

General Properties and Relation to Proteins :—The polypeptides like the free amino-acids contain the free amino and a free carboxyl group and hence, exist as *dipolar* or *Zwitter ions*. They are colourless compounds, soluble in water but insoluble in alcohol and acetone. They do not melt but decompose at about 200°C. They have a bitter taste like the proteins and are likewise precipitated in sulphuric acid solution by phospho-tungstic acid, tannic acid etc. They also give the biuret reaction like the natural proteins. They are attacked by nitrous acid with evolution of nitrogen; acids and alkalies hydrolyse them to free amino-acids. However, the enzymes that split proteins, do not attack poly-peptides under comparable conditions of experiments. Thus pepsin, trypsin and papain—from the green fruit of papaya, which hydrolyse proteins very readily, do not act on the synthetic or even the natural di-or tri-peptides. Recently, Bergmann has shown that the differences in response to the enzymes is due to the fact that natural proteins, unlike the synthetic or natural poly-peptides, do not contain free amino or free carboxyl groups. With peptides of the type R—CO—NH—CHR′—CO—NH—R″ which contain neither a free amino-group nor a free carboxyl group, the action of the enzyme is normal and such peptides are readily hydrolysed by the usual proteinases.

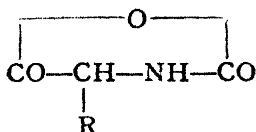
Thus these results indicate that the polypeptides are the close structural analogues of the natural proteins.

Synthetic proteins

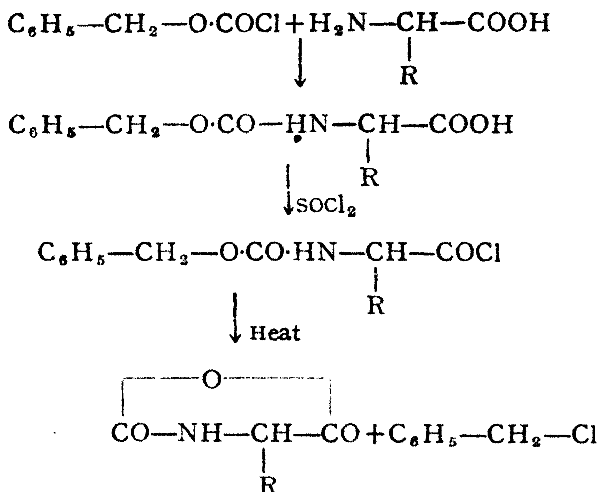
Recently, Woodward and Schramm have claimed to have synthesised protein molecules of minimum average molecular weight of several millions, and having the general structure:



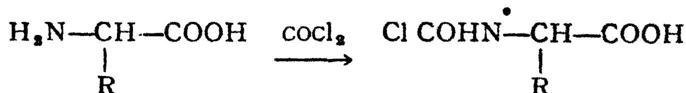
They have used an ionic chain polymerisation reactions: the monomers used are the anhydrides of N-carboxy-α-amino-acids:



The latter are also known as oxazolid 2·5 diones. They are prepared as follows :



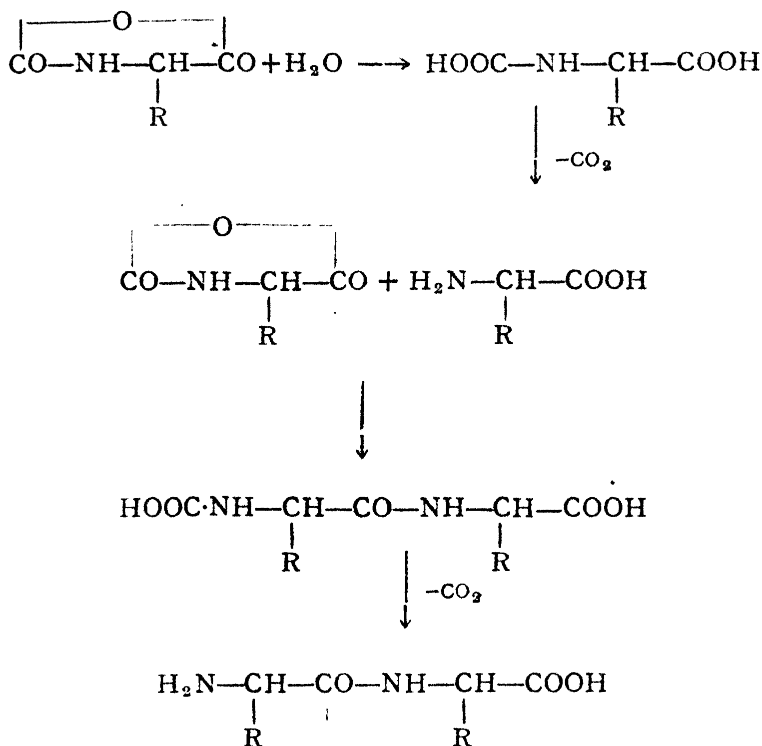
In another method, the oxazolid 2·5 dione is obtained directly from the α-amino acid and $COCl_2$.



which loses HCl on heating alone or with Ag_2O in acetone and gives the oxazolid, 2·5 dione (N-carboxy-anhydride).

The oxazolid, 2·5 diones are then polymerised: water or any compound HX, in which H is an active hydrogen atom, can act

as the initiator; the reaction which is a chain reaction, proceeds thus:



which reacts with another molecule of oxazolid 2·5 dione and the process is repeated several hundred times to give a polymer. In an actual experiment, the N-carboxy-*l*-leucine anhydride and the N-carboxyl-*dl*-phenyl alanine anhydride were co-polymerised in ordinary benzene, containing a small amount of water as the initiator. The solution became gradually viscous, as the reaction proceeded. After two weeks, at ordinary temperature, such a solution when cast, gave optically active, clear, tough and mechanically stable films. The material analysed for carbon and hydrogen as $C = 68{\cdot}6$ and $H = 8{\cdot}5$ per cent. Careful osmotic pressure measurements, of 0·5% and 0·75% benzene solution of the film in the sensitive Fuoss osmometer, gave values of Δh, not different from zero. These results indicate a minimum average molecular weight of several millions.

Attempts to synthesise proteins by the agency of an enzyme are also recorded. The enzyme papain has been shown to take part in the forging of a peptide linkage; and it is expected that eventually its use may lead to the synthesis of a protein like molecule.

Structural Theories for the Protein Molecule:—Dispite the large amount of analytical and synthetic work by Fischer and others, our knowledge of the structure of the protein molecule is meagre and empirical. Several theories of protein structure have been put forward and these have found more or less general acceptance. Some of them are:

(*a*) The polypeptide theory by Fischer.

(*b*) The diketo-piperazine theory by Abderhalden.

(*c*) The pyrrole theory by Troensegaard.

(*d*) The oxazole theory of Karrer.

(*e*) The cyclol theory of Wrinch.

(*a*) THE POLYPEPTIDE THEORY:—This theory has been advanced by E. Fischer. The acid hydrolysis of all natural proteins yields invariably a mixture of α-amino acids, which, therefore, must constitute the structural units of the complex proteins. The protein molecules are condensation polymers of the polyamide type originally contains relatively few *free* amino and carboxyl groups, the number of such groups goes on increasing as the hydrolysis progresses. It is, thus, evident that there are present in the protein molecule the amide ($-CO-NH-$) type of linkages, formed by the combination of the H_2N group of one amino acid molecule with the $COOH$ of another Further evidence pointing to the presence of the peptide linking is based on the following considerations:

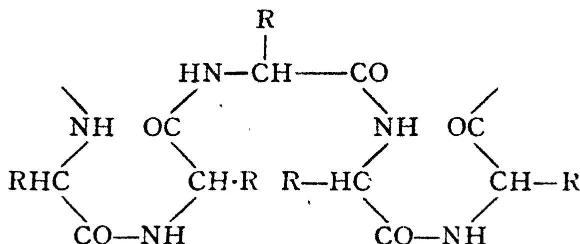
(*i*) Polypeptides (di, tri and tetra–) are frequently encountered with, when proteins are incompletely hydrolysed.

(*ii*) The synthetic polypeptides obtained by Fischer from optically active amino acids of the *l*-series, are hydrolysed by the enzymes present in the intestines.

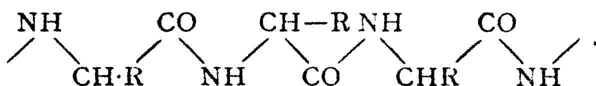
(*iii*) As in the case of the proteins, the hydrolysis of the synthetic polypeptides give rise to equal amounts of free amino and carboxylic groups.

(*iv*) The amide type of linking (CO—NH) is found in many natural products of great physiological importance. *e.g.* penicillin, folic acid, pantothenic acid etc.

The presence of the peptide linkage in the protein molecule is also indicated by physical evidence. Recent researches of Artburg and co-workers, embodying the results of X-ray analysis of the animal fibres have revealed that α-keratin—the protein constituent of all animal hairs, is built up of an *unstretched* chain—



On stretching, α-keratin is converted into β-keratin, which contains *stretched* peptide chain :



Such a formulation also explains the basic properties of hair and the process of denaturation of the proteins; the denatured form corresponds to the folded chain structure while the natural protein possesses the stretched chain form.

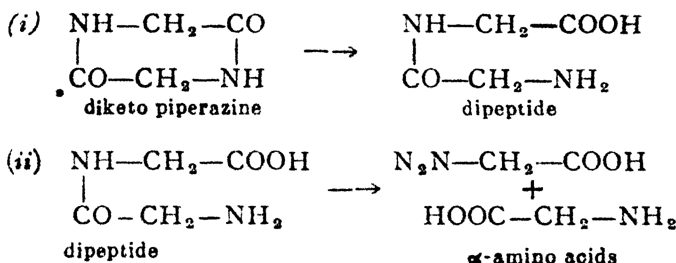
At some time, the peptide theory was challenged on the basis of the observation that the enzymes which hydrolyse proteins do not, under comparable conditions of experiment, attack the synthetic polypeptides. This objection has now been overruled by the investigations of Bergmann, who has shown that polypeptides not carrying free amino and free carboxylic groups, are readily attacked by the enzymes that decompose the natural proteins.

Recent work of Woodward and Schramm on the synthetic proteins (see p. 697), obtained by a chain polymerisation reaction constitutes decisive evidence in favour of the peptide theory. The structures proposed by L. Pauling for proteins are known as α-helix

in which each NH linkage of the peptide chain forms a NH←O bond with the nearby CO group of the next turn of the helix. This is in good agreement with the peptide theory of proteins.

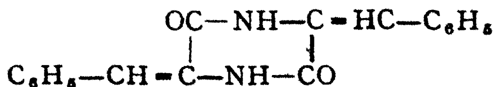
(b) THE DIKETO-PIPERAZINE THEORY :—According to this theory, put forward by Abderhalden, the protein molecule is built up of a number of piperazine complexes bound together by secondary valencies. He has claimed to have isolated a number of diketo-piperazines from proteins by mild chemical or fermentative disruption. He has chiefly employed methods of *oxidative* and *reductive* degradation. Further, the diketo-piperazines give colour reactions with picric and 3·5 dinitro benzoic acid, similar to the reactions given by most proteins and peptones with the same reagents. (Such reactions are not given by α-amino acids).

The formation of polypeptides and α-amino acids as the intermediate and final products respectively, of hydrolysis of proteins is then explained thus : the diketo-piperazine is capable of hydrolysis in *two* stages—



The stability of the protein molecule is satisfactorily accounted for by the presence of the *cyclic* diketo-piperazine ring in the molecule.

However, this hypothesis is based on purely analytical evidence only. So far, no synthetic evidence has been brought forward in favour of this hypothesis. The synthetic diketo-piperazines are not attacked by enzymes that decompose proteins. Secondly, if the diketo-piperazine ring were present, benzaldehyde would condense with the *methylene* groups to give the compounds :



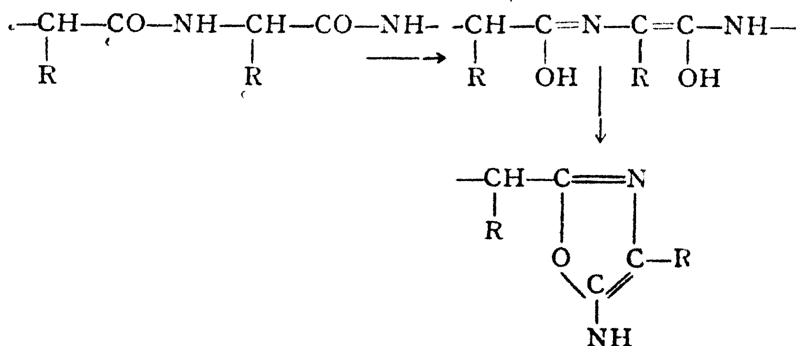
which, on reduction and subsequent hydrolysis, would yield phenylalanine, $C_6H_5-CH_2-CH(NH_2)-COOH$. Dakin, on the other hand, found that benzaldehyde condensation products of glycine-rich proteins are extremely stable towards hydrolysis :

Hence, this hypothesis must be regarded as only plausible and tentative, and more work has to be done both by the analytical and synthetical methods, before it meets with general acceptance.

(*c*) THE PYRROLE THEORY :—Troensegaard has subjected proteins to dry distillation. He has, thus, investigated the proteins of gelatin, blood, wheat and milk. In order to avoid the hydrolysis of the proteins by water, they were treated in *non-aqueous* solvents and as the proteins are very sensitive towards chemical reagents, methylation and acetylation of the molecules were resorted to. The acetylated proteins were then reduced with sodium and alcohol and finally, dry-distilled. Acidic and basic fractions were obtained which possess a hetero-cyclic character and chiefly a *pyrrole* nucleus. On the basis of these experiments Troensegaard has suggested that the protein molecule is probably built up of pyrrole-like compounds.

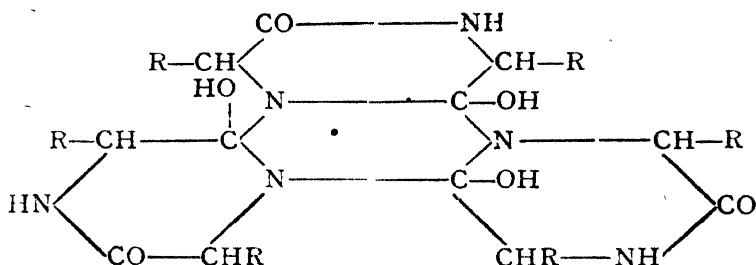
However, there is not *sufficient* experimental evidence as to the exact nature of the various fractions ; also no synthetic work of any importance has been carried out in this connection which would constitute more definite evidence for the structure of this type.

(*d*) KARRER'S OXAZOLE THEORY :—Karrer and collaborators showed that oxazole units can be formed by the dehydration of a segment of the polypeptide chain.



On the basis of the above considerations, they proposed that oxazole units may actually form a part of the protein molecule. They synthesised a number of oxazoles from a variety of acyl derivatives of amino acid esters, to see the possible occurrence of such compounds as units in the protein molecule. However, the results so far obtained have not been very encouraging.

(*e*) The Cyclol Theory of Wrinch.—According to this theory, the proteins are closed polypeptide chains. The resulting mosaics consist of hexagonal rings and is very complicated in structure. A simple example of the cyclol from six amino-acid residues is as follows:



Wrinch showed that more complex cyclol surfaces than the above, can be made to form three dimensional structures.

CHAPTER IX

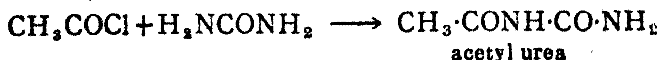
UREIDES AND THE PURINES

Introduction :—The chemistry of ureides and purine derivatives has been systematically investigated by Liebig, Wohler, Fischer. Uric acid found in urine, guano and the excrement of the reptiles, attracted the early attention of these researchers. Liebig and Wohler studied the decomposition of uric acid with oxidising agents and obtained many products which were assigned simple empirical formulas. It was Baeyer who clarified their complex relationships and showed that they were related to urea *i.e.* they were *ureides.* Ureides are compounds formed by the condensation of urea with acids and contain the amide type (*CO—NH*) of linking. They may be called *Acyl ureas*. Structurally, they belong to the class of acid amides.

Nomenclature and Classification :—The ureides have been classified according to (*i*) the *number* of urea residues present, or according to (*ii*) the structure of the ureide. Thus we have: (*a*) mono ureides and di-ureides, depending on the number of urea residues present in the ureide molecule. Or, the ureides are classified as (*i*) acyclic or open chain ureides and (*ii*) cyclic ureides, according to the open chain or closed chain structure of the ureide molecule. The cyclic ureides containing a six membered ring, are also regarded as derivatives of the fundamental type; pyrimidine or 1·3 diazine. Lastly as they are derivatives of acids, they may be derived from (*a*) mono basic acids, (*b*) α-and β hydroxy-or-halogeno mono basic acids and (*c*) di-basic acids.

Modes of Preparation :—A number of methods have been developed for the synthesis of ureides. A few typical ones are indicated below:

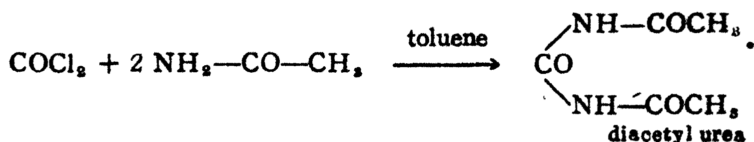
(*i*) Mono-ureide is formed by the action of acid chloride or acid anhydride or ester on urea in an inert medium or in pyridine.



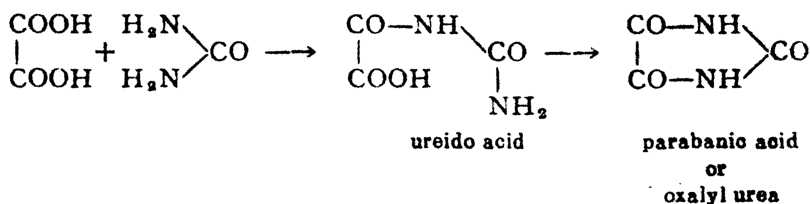
$$CH_3COCl + H_2NCONH_2 \longrightarrow \underset{\text{acetyl urea}}{CH_3{\cdot}CONH{\cdot}CO{\cdot}NH_2}$$

Only one acyl group can be thus introduced into the urea molecule. Such ureides are neutral and form no salts.

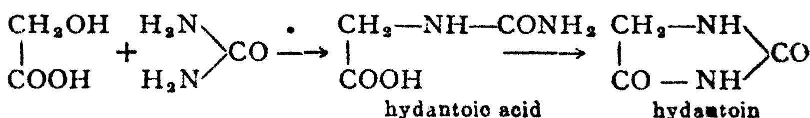
(*ii*) Diacyl-ureas are obtained by the action of $COCl_2$ on acid amides :



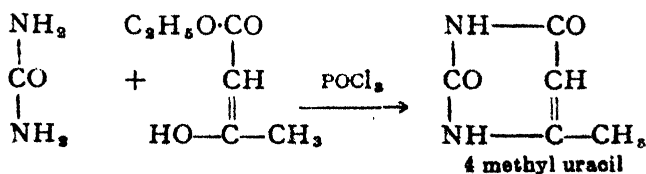
(*iii*) With dibasic acids, two kinds of derivatives, the *ureido* acids and *ureides* are possible ; when only one CO_2H group is involved in the condensation, ureido acids are formed and when both the COOH groups take part in the condensation, *cyclic* ureides are obtained. As the five and six membered rings are stable, cyclic ureides so far known are almost limited to those derived from oxalic and malonic acids and their derivatives. They are obtained by the condensation of urea with dibasic acids or their esters in presence of $POCl_3$ at 120° to 140°.



Hydroxy-monobasic acids which contain a hydroxyl and a carboxyl group give rise to ureido acids and ureides.



(*iv*) β-Ketonic acids or esters and β-aldehydic acids condense in a similar way with urea to yield cyclic ureides. Such ureides are called *uracils*.



Many of these uracils are present in plant nucleic acids of yeast, thymus and fish sperm.

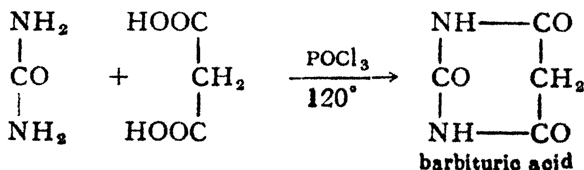
General Chemical Behaviour :—As acyl derivatives of urea, the ureides contain two types of linking: (*i*) the imide CO—NH—CO— linking and (*ii*) the other—NH—CO—NH—; the characteristic reactions of CO—NH—CO—linkings are: (*i*) the H of NH may be replaced by metallic atoms and subsequently by alkyl groups; the ureides thus dissolve in alkalies; (*ii*) with PCl_5 or $POCl_3$, the corresponding chloro derivative—N = C—Cl is obtained. On account of the presence of the NH—CO—NH grouping, the ureides on hydrolysis, are quantitatively broken down into urea and an organic acid. This reaction has great analytical value and is used to establish the constitution of the ureides.

The cyclic ureides derived from malonic acid contain the linking: CO—CH_2—CO, which carries a reactive methylene group. The H atoms of such an ureide can be replaced by alkyl groups, by Br atom or by NO and NO_2 group. The derivatives thus obtained are of great synthetic value.

Ureides

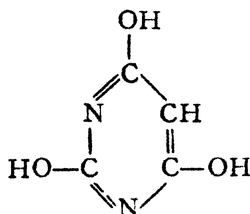
Many simple ureides are known which are closely related to uric acid; a few of them are readily obtained from the latter by oxidation. They can be classified as cyclic ureides and contain either a six membered ring or a five membered ring. But they are not classified as hetrocycles as they are readily decomposed. The six membered ring ones are: barbituric acid, alloxan, violuric acid, dialuric acid, uramil and uracil. The five membered ones include: parabanic acid, hydantoin and allantoin.

BARBITURIC ACID: This compound is of great interest as Fischer obtained uric acid from pseudo-uric acid, one of its simple derivatives. It is derived from malonic acid and urea and hence is called malonyl urea.



barbituric acid

Babituric acid is also obtained by heating urea with ethyl malonate in presence of $NaOC_2H_5$ at 125°. Barbituric acid has been sometimes formulated as a pyrimidine derivative. It is white powder mp. 245°. It is stronger than acetic acid.



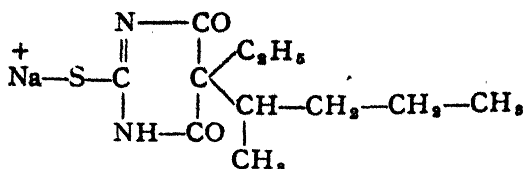
Such a formulation shows the resemblance of barbituric acid to phenolic compounds. However, the compound is not at all aromatic, but is completely hydrolysed to urea and malonic acid. The true aromatic compounds are more stable. Hence the rectangular formulation is to be preferred. But the above enolic formulation accounts for the great acidity of the molecule.

The—CH_2—group in the barbituric acid is very reactive and gives rise to alkyl, aryl or mixed alkyl-aryl derivatives. They are therapeutically very important and find extensive use as soporifics. They are called "Barbitals," or Barbiturates. They are obtained by the condensation of the appropriately substituted malonic ester with urea in presence of $NaOC_2H_5$. Some of the well-known are:

Veronal	→	5, 5 diethyl-barbituric acid
Neonal	→	5 ethyl-5-n butyl barbituric acid
Luminal	→	5 ethyl-5-phenyl barbituric acid
Amytal	→	5-ethyl-5-isoamyl barbituric acid
Dial	→	5-5, di-allyl barbituric acid

Veronal is a solid m. p. 191°: the Na-salt which is more soluble is used. It acts on the central nervous system and exercises sedative action.

Pentothal-sodium, is another important modern drug. It is the Na-salt of 5, 5′ (1 methyl-butyl-ethyl-thiobarbituric acid.



A dilute solution (2·5—5%) is used intravenously to produce anæsthesia.

The ureides mentioned earlier are all inter-related to one another and to barbituric acid, which can be called the 'key' compound. These relationships are outlined schematically as follows:

```
NH——CO                      NH——CO
|    |         HNO2         |    |
CO   CH2     ———————→       CO   C=NOH
|    |                      |    |
NH——CO                      NH——CO
barbituric acid             violuric acid

   HNO3 \ O2            ↗ NH2OH            | Na + alcohol
         ↘             /                   ↓

NH——CO                  NH——CO          NH——CO
|    |          H2      |    |          |    |
CO   CHOH   ←————————   CO   CO         CO   CH—NH2
|    |       Zn + HCl   |    |          |    |
NH——CO                  NH——CO          NH——CO
dialuric acid           alloxan         uramil
                           | HI            |
                           ↓               ↓
                                        uric acid

        / electrolytic \
           reduction    ↘
NH——CO                  NH——CH2                 H2N
|    |  (excess)Br2     |    |      1 Br2        |
CO   |  ←———————————    CO   |    ————————→      CO
|    |      H2O         |    |     2 urea        |
NH——CO                  NH——CO                  NH—CH—NH\
parabanic acid          hydantoin                   |      CO
   ↑                                                CO—NH/
   └——————————————————————————————————————————————┘
                     HNO3                         allantoin
```

VIOLURIC ACID is thus iso-nitroso-barbituric acid. Its salts are intensely coloured; Mg, Pd and Ba-salts are purple. The appearance of colour is probably due to crossed double or conjugated systems of double bonds present in the molecule.

URAMIL :—It is 5-amino barbituric acid and is obtained by the reduction of iso-nitroso barbituric acid or violuric acid.

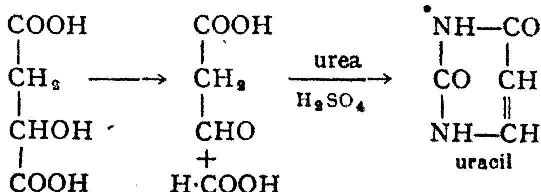
ALLOXAN :—This is also a product of oxidation of uric acid. On hydrolysis, it gives urea and meso-oxalic acid; hence it is meso-oxalyl urea. It behaves as a typical ketone; the central CO group is very reactive; it gives an addition product with $NaHSO_3$; it forms an oxime which is identical with violuric acid. On reduction, dialuric acid is formed.

PARABANIC ACID :—It is oxalyl urea; and hence is obtained by the condensation of urea and oxalic acid. It is also obtained as a product of oxidation of uric acid. It is soluble in alcohol and water. With alkalies it yields salts. It is a crystalline compound mp. 244°.

HYDANTOIN :—This is the cyclic ureide derived from urea and α-hydroxy acetic acid. It is also obtained by the electrolytic reduction of parabanic acid; with alkalies, it gives salts.

ALLANTOIN :—It is a decomposition product of uric acid under alkaline conditions; it is a urea derivative of hydantoin and is obtained by brominating hydantoin and condensing it with urea.

URACIL is a cyclic mono-ureide obtained by treating a mixture of urea and malic acid with fuming H_2SO_4. The course of the reaction is:



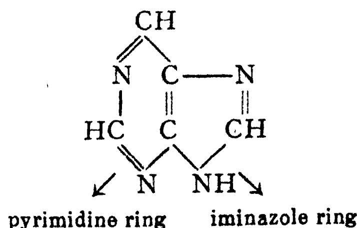
Thymine, the constituent of the nucleic acid of Thymus is 5-methyl-uracil.

Purines

Introduction :—The purines include uric acid and the xanthine bases, which represent the products of animal metabolism. Uric acid was first isolated by Scheele in 1776 from urinary calculi-stone in the bladder. It is now found in human urine and in the excreta of birds, snakes and animals; the guano of the south sea islands contains as much as twenty-five per cent of uric acid by weight. Xanthine, theobromine, caffeine, hypoxanthine, guanine etc., which together constitute the xanthine bases are also found widely distributed in the animal and plant kingdoms. Xanthine is a constituent of many animal tissues; theobromine and caffeine are present in cocoa beans; tea and coffee contain small quantities of theophylline in addition to caffein; guanine and adenine are the primary products of the hydrolysis of *nucleic acids.*

General Composition and Structure :—Uric acid and the xanthine bases are complex organic compounds, which contain carbon, hydrogen, oxygen and nitrogen. Their structural chemistry has been thoroughly elucidated by the pioneer researches of Emil Fischer and his students. The constitution of every one of these compounds has been arrived at both by degradation methods and by synthesis.

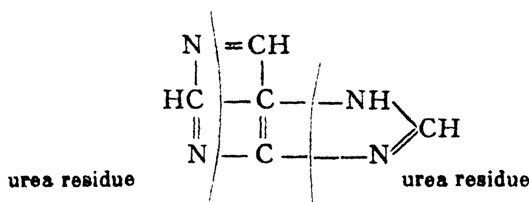
Structurally, they have been proved to be derived from a parent compound which Fischer has called *purine.* Purine contains a condensed hetero-cyclic system built up of a pyrimidine ring and a glyoxaline (iminazole) ring and has the structural formula, the hexagonal structure).



This formulation clearly brings out the aromatic character of some of these purine derivatives. The purines condense with diazonium salts to form coloured compound with the grouping Ar—N = N —C—. Such compounds on reduction give an amino purine derivative.

The relatively greater solubility of the pürines, in water is to be attributed to the presence of many ammono-aldehyde groups : CH=N—.

Another formulation of purine which is most commonly adopted is its representation as a cyclic di-ureide system (rectangular formula)



The purines are the *di-ureides,* formed by the condensation of the two molecules of urea with a hydroxy acid. On hydrolysis they invariably give *two molecules of urea.* This conception of the purines as ureides has great synthetic significance. All the synthetic work in this field has been based on and related to the formulation of these compounds as urea derivatives. All the syntheses of the purines start from urea.

Classification and Nomenclature :—The purines, *e. g.* uric acid, xanthine, caffeine, guanine etc. are the hydroxy or amino derivatives of purine. Thus, we have :

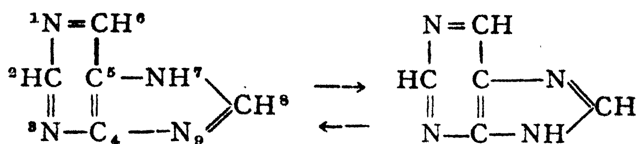
(*a*) *Oxy-purines* :—The typical members are hypo-xanthine, xanthine and uric acid of which the most important and the best known is uric acid. Theobromine, theophylline and caffeine are the methyl derivatives of the hydroxy–purine, xanthine.

(*b*) *Amino-purines* :—Adenine and guanine, the products of hydrolysis of nucleic acids are the typical amino-purines.

(c) Thio-purines :—They contain *SH* groupings and are purely synthetic products.

The nomenclature most commonly adopted is the one due to E. Fischer. The purine is represented as a di-ureide containing the carbon-nitrogen skeleton as shown above. The position of the substituent hydroxy, amino or methyl group is indicated by a number. The following is the system usually adopted for numbering

the carbon and nitrogen atoms in the compound for naming the derivatives :



The two forms are tautomeric and cannot be distinguished from each other by any chemical method.

Uric Acid :—This is the best known purine and is a nitrogenous product of animal metabolism. Hence, it is found in human urine and in the excreta of birds, reptiles and snakes. The chief source of uric acid is *guano*, in which it is present as the *ammonium salt* to the extent of 25 per cent.

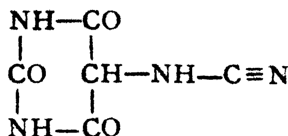
Isolation :—The guano is boiled with con. $NaOH$ till all the ammonia is expelled. The hot liquid is then filtered into HCl acid; on cooling, the acid crystallises out and is dried at 100°.

Uric acid is a white granular powder insoluble in cold water but sparingly soluble in hot water. It is a weak dibasic acid and gives two series of salts (mono and di-) which also are difficultly soluble. It does not melt; it is identified by the murexide test.

CONSTITUTION :—The molecular composition of uric acid is $C_5H_4N_4O_3$. Its structural formula has been elucidated through the extensive researches of Liebig, Wohler, Baeyer and Fischer. The first elaborate investigation was carried out by Liebig and Wohler in 1838. They prepared and examined a number of derivatives of uric acid, but could not throw any light on their constitution. It was left to Baeyer to trace and unravel the relationships existing among these many derivatives. He regarded that all these compounds were derived from barbituric acid or malonyl urea, a compound with the composition $C_4H_4N_2O_3$. On hydrolysis, it breaks down into (*a*) malonic acid and (*b*) urea. Later on, Grimault synthesised barbituric acid by heating together malonic acid and urea in presence of phosphorus oxychloride.

Baeyer then made several attempts with partial success only, to achieve a synthesis of uric acid. Starting from barbituric acid he obtained a compound with the composition $C_5H_6N_4O_4$, which

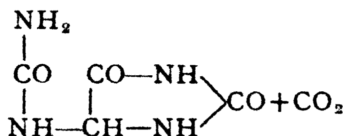
contains one H_2O molecule more than uric acid. He called it pseudo-uric acid. He however, could not succeed to dehydrate it to uric acid to which he assigned the structure (Barbityl-cyanamide).



It was Fischer who achieved success by converting the pseudouric acid into uric acid by the action of molten oxalic acid, and also established its structure. The above results, however, did not give an insight into the constitution of the uric acid molecule. The structural chemistry has been elucidated by the study of results of oxidative and hydrolytic reactions of the molecule, carried out under different experimental conditions.

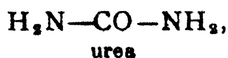
OXIDATION REACTIONS :—Strecker has investigated the action of different oxidising agents on uric acid. He found that :—

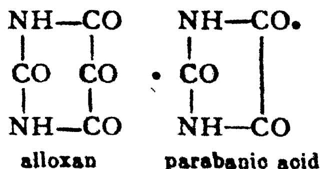
(*a*) Oxidation of uric acid with *alkaline potassium permanganate* gives CO_2 and allantoin, which has the structure :—



The presence of iminazole ring is indicated.

(b) With acid $KMnO_4$, uric acid is degraded into :

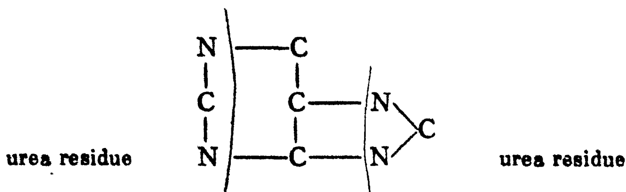




(*c*) Nitric acid converts uric acid into alloxan and urea; some parabanic acid is also formed.

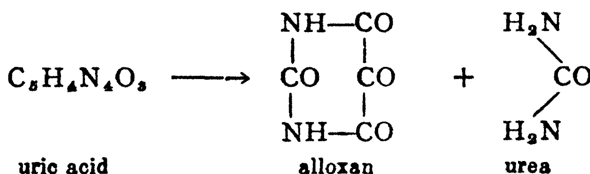
As uric acid is degraded oxidatively into alloxan and parabanic acid, it must contain the six-membered alloxan ring and the five

membered iminazole ring. These two rings together contain seven carbon and four nitrogen atoms. Uric acid, $C_5H_4N_4O_3$, however, contains only five carbon atoms. It, therefore, follows that two carbon atoms are common to both the hetero rings. Hence, the probable atomic framework in uric acid molecule is :—

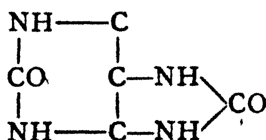


An inspection of the above skeleton further reveals the presence of two urea residues, *i.e.* uric acid must be a di–ureide. This conclusion is completely confirmed by results of hydrolytic reactions.

HYDROLYTIC REACTIONS :—With chlorine water, uric acid is decomposed into alloxan and urea :—



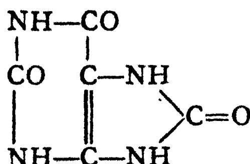
Both hydrolysis and oxidation have occurred and the results definitely prove that uric acid molecule contains an alloxan ring fused with a urea residue, because, the total number of carbon and nitrogen atoms in the two decomposition products is the same as in the original uric acid molecule. Further, as alloxan is a mono-ureide, it follows that uric acid is a di-ureide. Hence the formula for uric acid may be further evolved to the following one :



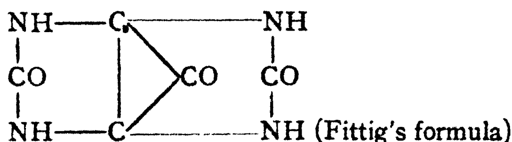
The presence of four imino (NH) hydrogen atoms, is indicated by the formation of a tetra-methyl uric acid derivative, which on boiling with HCl acid yields four molecules of CH_3NH_2. The

formation of alloxan, indicates that there is probably a *CO* group in position 6.

Hence, uric acid must be represented by the formula :—



Quite early in the history of the chemistry of uric acid, two different formulas had been proposed by Medicus and Fittig each. Medicus' formula was the same as given above, and is an unsymmetrical one. Fittig had formulated the acid by a perfectly symmetrical structure :—

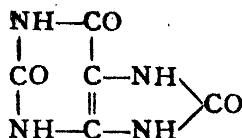


Both the formulas indicate the presence of four imino groups as required by the existence of a tetra-methyl uric acid. Further, according to both the formulas, uric acid is a di-ureide. However, in Medicus' formula, the two urea residues are unsymmetrically arranged while they are symmetrically placed in the Fittig's formula. The final choice of the former structure for uric acid rests on the following experimental evidence :—

Uric acid can be converted into four isomeric mono-methyl uric acids. Two of them on treatment with chlorine water give alloxan and methyl urea ; the other two give under the same conditions, methyl alloxan and urea. These results therefore, show that in two of them the urea residue outside the alloxan ring is methylated, while in the other two, the alloxan ring itself has suffered methylation. These results thus clearly show the unsymmetrical arrangement of the two urea residues in the uric acid molecule. In the Fittig's formula, the two urea residues are symmetrically placed in the molecule.

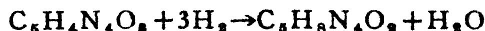
Lastly, Fischer could isolate four isomeric mono-methyl uric acids. Fittig's formula cannot yield more than one mono-methyl

derivative. Hence, uric acid must be assigned the unsymmetrical di-ureide formula :—



This structure for uric acid was also indirectly confirmed by Fischer's illuminating researches on caffeine (*q. v.*).

The above formula carries a double bond in between C_4 and C_5. The presence of this double bond is indicated by the following reaction. Uric acid, on electrolytic reduction gives purone :

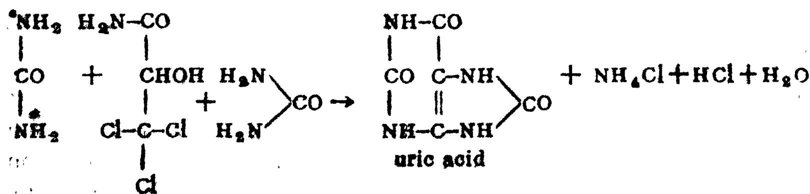


$$C_5H_4N_4O_3 + 3H_2 \rightarrow C_5H_8N_4O_2 + H_2O$$

i. e. Six H atoms are involved in the reaction ; of these, two H atoms are used up to remove one oxygen atom and two to replace it, the remaining two must have been used up to saturate the double bond.

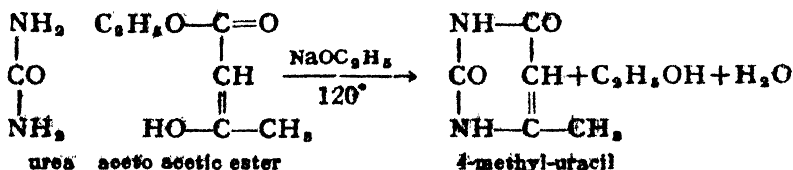
SYNTHESES OF URIC ACID :—The above constitution of uric acid has been finally confirmed by numerous syntheses. Some of the most important ones either from the theoretical or the preparative stand-point will be discussed here.

1. *Horbaczewske's synthesis :*—This is the first synthesis of uric acid. Trichloro-lactamide is fused with urea ; schematically, we have :—

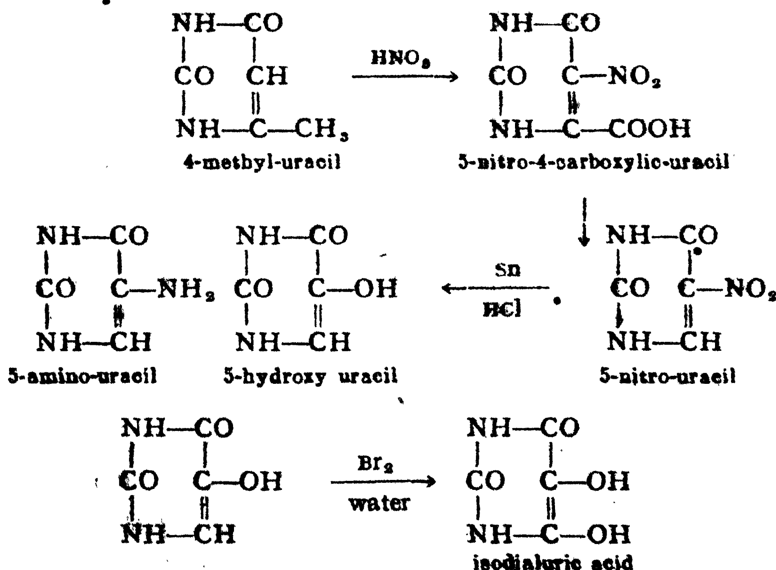


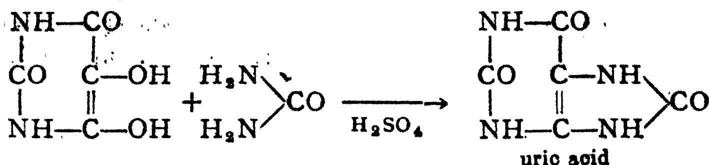
The yield of the acid is poor ; and secondly, the reactions involved do not reveal the exact constitution of the molecule formed. A more unambiguous synthesis which gives insight into the structure of the molecule is due to Behrend and Roosen.

1. *Behrend and Roosens' Synthesis* :—The starting-point is urea; it is condensed with aceto acetic ester to form 4-methyl-uracil :—



On treatment with fuming nitric acid, 4-methyl-uracil is converted into 5-nitro-4-carboxylic-uracil. The changes involved in the above conversion are both oxidation and nitration of the molecule by nitric acid. On heating, the 5-nitro-carboxylic-uracil loses carbon dioxide and forms 5-nitro-uracil. The latter is then reduced with tin and hydrochloric acid to a mixture of 5-amino and 5-hydroxy-uracil. Probably, the 5-hydroxy-uracil is formed by the hydrolysis of the amino compound. The 5-hydroxy-uracil is then oxidised by bromine water to form 4-5-dihydroxy-uracil (iso-dialuric acid). The latter is condensed with urea in presence of con. sulphuric acid to give uric acid. Schematically the above reactions are expressed as follows :



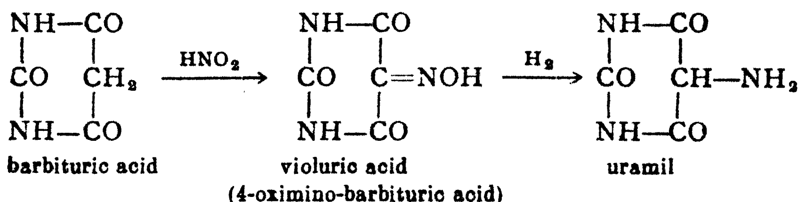


This synthesis is of great theoretical interest as it is quite straightforward and unambiguous, and thus, reveals the exact structure of uric acid (the position of the double bond is definitely revealed).

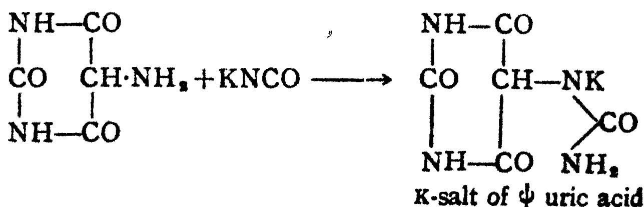
There are two more synthetic methods, one developed by Fischer and the other by Traube: both of them are of great preparative value.

3. *Fischer's synthesis* :—This method was first used by Baeyer to synthesise uric acid but he could only obtain pseudouric acid. Fischer and Ach, later on, succeeded in converting the latter into uric acid by fusing it with oxalic acid or by heating it with 20 per cent hydrochloric acid. The starting-point is barbituric acid; the different steps involved are :—

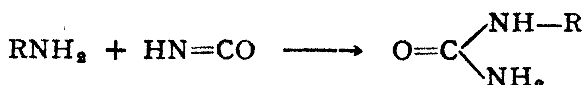
Barbituric acid is converted into 5-amino-barbituric acid (uramil) by the action of nitrous acid and subsequent reduction :—



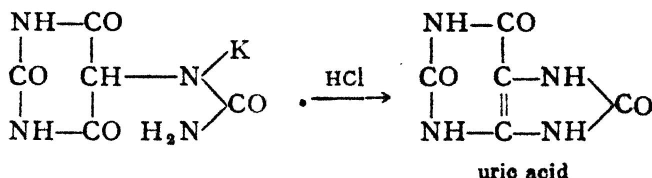
The uramil or 5-amino-barbituric acid is boiled with an aqueous solution of potassium iso-cyanate to form pseudo-uric acid :—



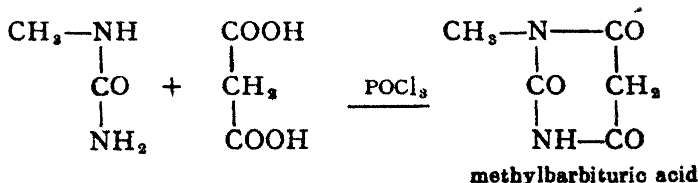
(The above reaction is a special case of the important method of preparing substituted (alkylated) urea derivative by the inter-action between an amine and iso–cyanic acid or its alkali salts :—



When fused with oxalic acid or heated with 20 per cent hydrochloric acid, pseudo–uric acid is changed into uric acid. Elimination of one molecule of water takes place with cyclisation of the iminazole ring :—



This synthesis can be extended to the preparation of methyl uric acids. Methyl uramils can be obtained starting from methyl ureas and malonic acids. Thus, we have :—

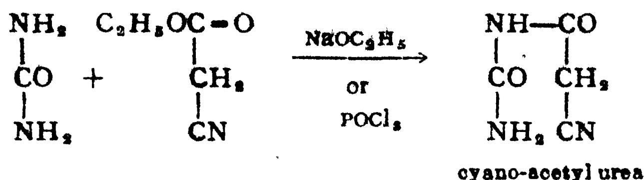


The latter on treatment with HNO_3 and subsequent reduction with ammonium sulphide gives methyl uramil. The methyl uramil can then be converted into the corresponding methyl uric acids, by heating with potassium iso–cyanate and subsequent boiling with 20 per cent hydrochloric acid.

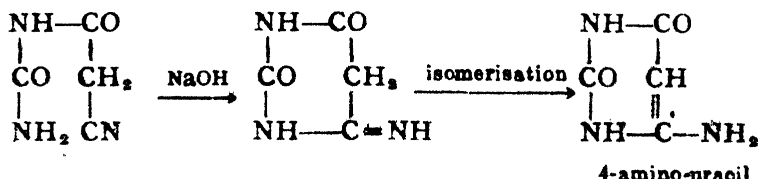
4. *Traube's synthesis* :—This synthesis is a more general one and of wider application. It has been fruitfully extended to the preparation of all purine derivatives. It is also simple and elegant. The starting materials are urea and cyano–acetic acid or ester.

The various steps in the synthesis can be formulated as below :—

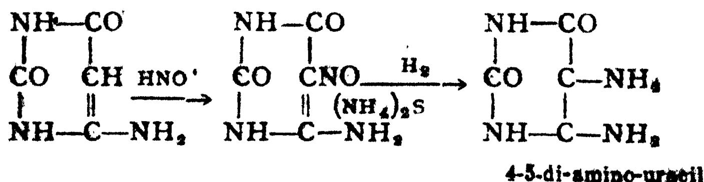
Formation of cyano-acetyl urea :—Urea is condensed with cyano-acetic acid or its ester in presence of mild alkali to form cyano-acetyl urea (Phosphorus oxy-chloride is also used for the condensation).



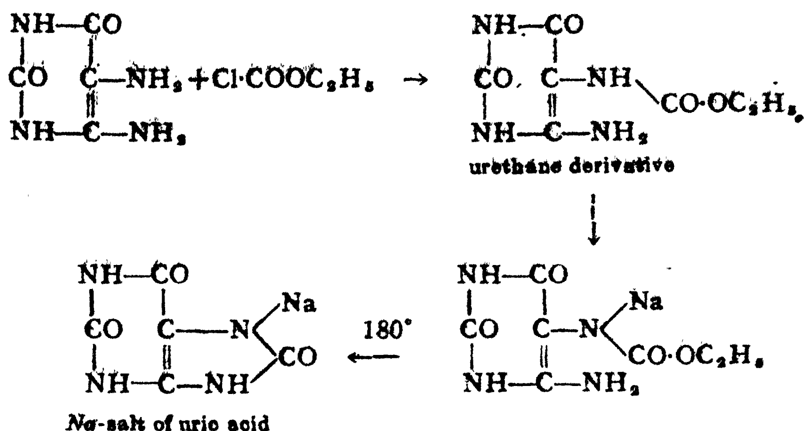
(*b*) *Formation of 4-5-diamino-uracil* :—Cyano-acetyl urea is treated with sodium hydroxide when cyclisation is effected and 4-amino-uracil is formed.



The latter is then converted into, 4–5-di-amino-uracil by the action of nitrous acid and subsequent reduction with ammonium sulphide :—



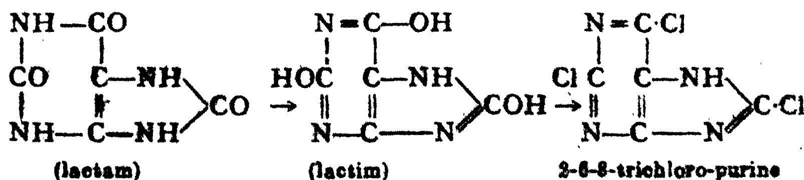
(c) *Formation of uric acid* :—The diamino-uracil is finally condensed with chloroformic ester to form a urethane derivative, the sodium salt of which on heating to 180°, is converted into the sodium salt of uric acid ; the yields are good.



Similarly, starting with methyl ureas it is practicable to synthesise the corresponding methyl uric acids. Replacements of urea by thio-urea or guanidine would result in the formation of thio-uric acids or amino-purines respectively. Lastly, with slight modifications this synthesis can be employed for the preparation of the xanthine bases (*q.v.*); uric acid is also obtained by fusing 4-5 diaminouracil with urea.

Reactions of uric acid :—

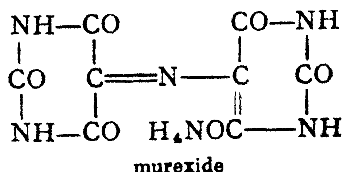
(*a*) With $POCl_3$ or PCl_5, uric acid is converted into chloro derivatives. At lower temperature, positions 2 and 6 are attacked; it is only at high temperature, that position 8 is attacked (with methyl uric acids, $POCl_3$ or PCl_5, preferentially attack position 8). The final product of chlorination of uric acid with $POCl_3$ or PCl_5 is 2, 6, 8-trichloro-purine. In this reaction, the acid behaves as if it contained three hydroxyl groups in molecule, in other words uric acid exists in two isomeric forms, *i.e.* the lactam and the lactim forms. It is the latter that reacts with the phosphorus halides to give the trichloro-purine derivative :—



Independent evidence for the observed tautomerism of uric acid has been obtained from the study of the absorption spectrum of the molecule. A solution of the free acid exhibits strong absorption (indicating the presence of centres of unsaturation), while the completely methylated uric acid molecule, in which the hydrogen atoms are substituted by methyl groups, shows no such absorption because in the case of the methylated uric acid, the hydrogen atoms capable of migration and hence, giving rise to the unsaturated grouping $N=C$ are not present.

Trichloropurine is a crystalline compound which decomposes at 184–186°, on account of the great activity of its halogen atoms, it constitutes the most fruitful source material for synthesis of all important purine derivatives. The order of activity of the halogen atoms is $6 > 2 > 8$.

(*b*) The murexide reaction is a colour test for uric acid. It consists in evaporating a small quantity of uric acid with a small amount of nitric acid and treating the residue with ammonia, when a deep-violet colouration is produced. The latter is ascribed to the formation of murexide, the ammonium salt of purpuric acid. It has been formulated as:



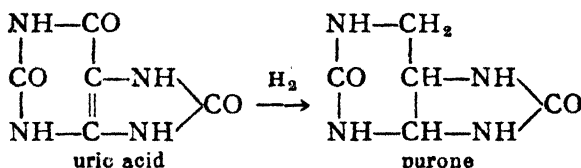
murexide

(*c*) *With alkalies* :—Uric acid, on account of the presence of imido (NH) groups, behaves like a weak acid towards alkalies, with the formation of metallic salts. It is only a dibasic acid, the hydrogen atoms in positions 3 and 9 being replaced by metallic atoms. Of the two hydrogen atoms, the former is more strongly acidic; the order of decreasing acidity of the H atoms is 3, 9, 1 and 7. The metallic salts of uric acid are converted into methyl uric acids by the action of methyl iodide.

(*d*) *Alkaline oxidation* :—Alkaline oxidation breaks down uric acid chiefly into allantoin. The oxidising agents commonly used are potassium permanganate, hydrogen peroxide and potassium persulphate in alkaline media.

(*e*) *Acid oxidation* :—In acid medium, uric acid is chiefly decomposed into alloxan and urea. Thus nitric acid or chlorine water oxidises uric acid to alloxan and urea. This decomposition has been of immense aid in the elucidation of the structure of uric acid and other related purine derivatives.

(*f*) *Reduction reactions* :—On electrolytic reduction, in sulphuric acid solution with lead cathode, uric acid is converted into a desoxy-derivative, purone.



(*g*) *Alkylation of uric acid* :—Alkylation of uric acid has been carefully investigated by Fischer as the alkylated uric acid can usefully serve as starting materials for the syntheses of xanthine bases. The alkylation has been accomplished in a number of ways. The important procedures employed are :—

(1) Action of methyl iodide on the silver or lead salt of uric acid.

(2) Action of dimethyl sulphate in presence of dilute sodium hydroxide.

(3) Action of diazomethane.

(4) Action of methyl iodide and alkali.

The position taken up by the methyl radicals, depends on the nature of the reagent used. Thus, when the lead or silver salt is used, uric acid is methylated successively in positions 3 and 9; with dimethyl sulphate and aqueous alkali, the hydrogen atoms in even positions 1 and 7 are substituted by methyl radicals. Diazomethane, on the other hand, is found not to methylate further than the 3–9 –dimethyl uric acid. The following methyl uric acids have been, thus, obtained :—

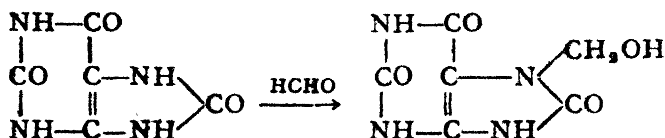
(*a*) Mono–methyl derivatives: 3–methyl uric acid; 9–methyl uric acid.

(*b*) Dimethyl derivatives: 1-3-di-methyl uric acid, 3-9-dimethyl uric acid, 7-9-di-methyl uric acid.

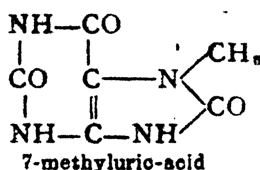
(c) Trimethyl derivatives : 1-3-7-tri-methyl uric acid, 3-7-9-tri-methyl uric acid.

(d) Tetra-methyl derivatives : 1-3-7-9 tetra-methyl uric acid.

There is another method of alkylation which, however, is of limited application only. Uric acid is heated with formaldehyde, to give 7-hydroxyl-methyl derivative.



On reduction with concentrated hydriodic acid it is converted into 7-methyl uric acid.



7-methyluric-acid

Xanthine Bases

A number of naturally occurring nitrogenous basic compounds are known, which are closely related to one another and to uric acid. They are the products of animal and plant metabolism.

They are found distributed as follows :—

xanthine	urine, animal organism
theobromine	cacao butter, cocoa beans
theophylline	,, ,,
caffeine (theine)	coffee or tea or kola nuts
guanine	guano
adenine	nucleic acids, fungi, muscles, liver etc.

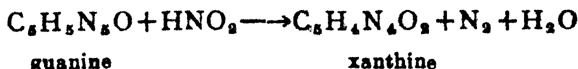
From the earliest times, it was suspected that guanine and xanthine were related to uric acid on account of the similarity in their molecular compositions :—

$C_5H_4N_4O_3$ uric acid,

$C_5H_5N_5O$ guanine,

$C_5H_4N_4O_2$ xanthine.

Later on, Strecker converted guanine into xanthine by the action of nitrous acid :—



$$C_5H_5N_5O + HNO_2 \longrightarrow C_5H_4N_4O_2 + N_2 + H_2O$$

guanine — xanthine

These results indicate that guanine contains an amino group which is replaced by an hydroxyl group to form xanthine.

Further, Strecker explained the inter-relationships existing between caffeine, theobromine, theophylline, and xanthine. Their molecular compositions are :

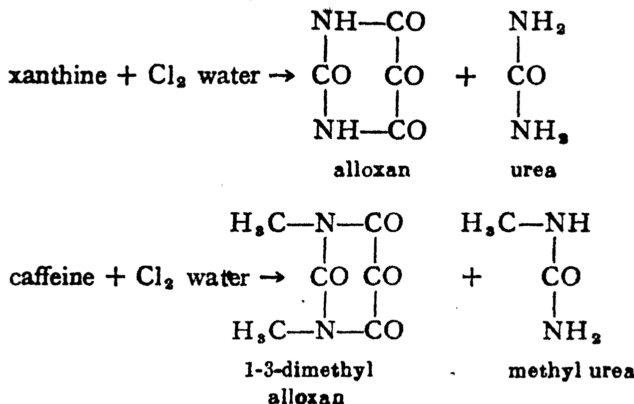
Xanthine	$C_5H_4N_4O_2$
Theobromine	$C_7H_8N_4O_2$
Theophylline	$C_7H_8N_4O_2$
Caffeine	$C_8H_{10}N_4O_2$

He showed that theobromine and caffeine are the di-methyl and tri-methyl derivatives respectively of xanthine; accordingly he converted theobromine into caffeine by the action of methyl iodide on the silver salt of theobromine but could not succeed in the conversion of xanthine into theobromine.

STRUCTURAL RELATIONSHIPS OF XANTHINE BASES :—Fischer has carried out elaborate investigations which have led to the complete solution of the problem of the structures of the xanthine bases and their relationship to uric acid. Indirectly, these studies have also confirmed the constitutional formula adopted for the uric acid. These investigations include new analytical and synthetical methods developed by Fischer.

Analytical methods :—Many of the xanthine bases contain N-methyl groups. The exact number of these groups has been determined by the Herzig's method. Theobromine and caffeine were shown to contain two and three methyl groups. Thus, Fischer could establish the genetic relationships existing between xanthine, theobromine and caffeine.

Oxidation of these bases with chlorine water, has yielded very fruitful results in the elucidation of their structures. Thus, we have :—



cf. uric acid + Cl_2 water → alloxan + urea

These results, therefore, reveal the positions of the methyl groups and the nature of the carbon-nitrogen framework present in the xanthine bases.

Synthetical methods :—Fischer then devised and employed a number of synthetic methods to elucidate and confirm the structural relationships obtaining between these bases. The following are the most important procedures :—

(*a*) Preparation of methyl derivatives of uric acid : these were obtained by (*i*) the action of methyl iodide on the silver or lead salt of the acid, (*ii*) the action of di-methyl sulphate in presence of dilute sodium hydroxide and (*iii*) the starting with methyl ureas.

The positions taken up by the methyl groups depend on the conditions of experiment. The following methyl derivatives were then obtained :—

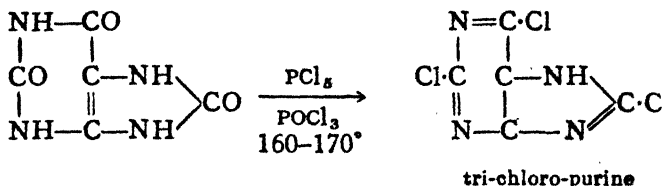
two mono-methyl uric acids ; (3 ; 9) ;

three di-methyl uric acids ; (1-3 ; 3-9 ; 7-9) ;

two tri-methyl uric acids ; (1-3-7 ; 3-7-9) ;

one tetra-methyl uric acid ; (1-3-7-9).

(*b*) Conversion of uric acid or the methyl uric acids into highly reactive halogen compounds. This was effected by the action of phosphorus halides. Uric acid was, thus, converted into tri-chloro-purine:



tri-chloro-purine

Similarly, 1-3-7-tri-methyl-uric acid was changed into 8-chloro-caffeine which was finally reduced to caffeine. This conversion readily establishes the structural relationship of caffeine to uric acid. 2-6-8-tri-chloro-purine contains reactive halogen atoms that could be replaced by hydroxyl, amine, ethoxy or hydrogen atom. Fischer successfully employed this compound to synthesise purine, xanthine, hypoxanthine, adenine and guanine, and thus, established their genetic relationships.

We shall now discuss in detail the application of the foregoing methods to the elucidation of the constitutional formulas of the xanthine bases.

CAFFEINE (THEINE):—This xanthine derivative occurs in leaves and beans of coffee, in the kola nuts and in tea leaves in small quantities. A cup of tea or coffee contains about 100 to 150 mg. of caffeine which is the therapeutic dose.

It is the most important of the xanthine bases, and is obtained in large quantities from uric acid; the latter is first methylated with $(CH_3)_2SO_4$ and alkali to give the tetramethyl derivative, which is converted into 8-chloro-caffeine by the action of $POCl_3$ at 160°. Reduction of the chloro-caffeine with HI in presence of PH_4I at 0° gives caffeine quantitatively. It can also be extracted from tea leaves.

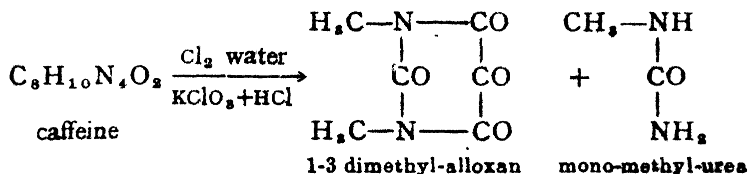
At present caffeine is commercially obtained from xanthine. The latter is first methylated and chlorinated to give 8-chloro caffeine which is reduced to caffeine with HI.

Caffeine forms long silky needles m. p. 232°; it possesses a bitter taste; it is a weak base and its salts are readily decomposed by water. It is a useful heart stimulant and a diuretic, and is used in the form of its citrate.

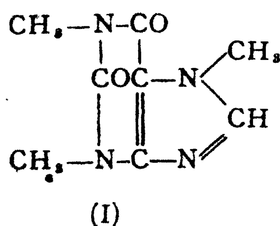
Structure:—The molecular composition of caffeine is $C_8H_{10}N_4O_2$. Its structural formula is based on the classical researches of Fischer.

(*a*) Herzig determination, *i. e.* heating with con. HI, to 200° indicates the presence of *three methyl* groups;

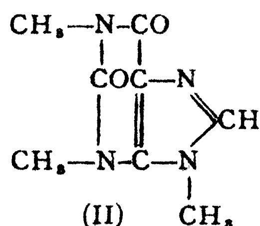
(*b*) On oxidation with chlorine water, it is converted into one molecule of 1-3-di-methyl-alloxan and one molecule of mono-methyl-urea :—



These results establish the position of two of the three methyl groups and also indicate that caffeine contains an alloxan ring, fused with a mono-methyl-urea molecule. Hence, caffeine may be (I) or (II) :—



(I)

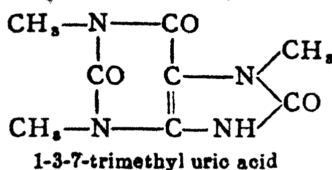


(II)

Caffeine, on drastic oxidative degradation givess-dimethyl-oxamide as one of the products of decomposition. This favours the formula (I), because: formula I carries the grouping: CH_3—N—C—C—N—CH_3, which is not present in formula II.

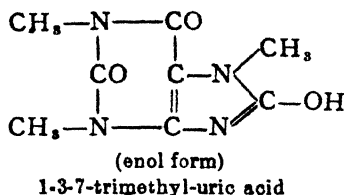
(*c*) Lastly, with chlorine in inert medium, caffeine is changed into mono-chloro-caffeine. The latter, on treatment with alcoholic potash and subsequent warming with dilute hydrochloric acid, gives

hydroxy-caffeine. The latter is identical with 1–3–7–tri-methyl-uric acid, which has been assigned the structure:—



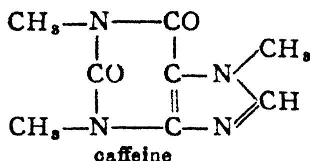
1-3-7-trimethyl uric acid

Hence, hydroxy-caffeine must possess the same structure as its enolic form:—



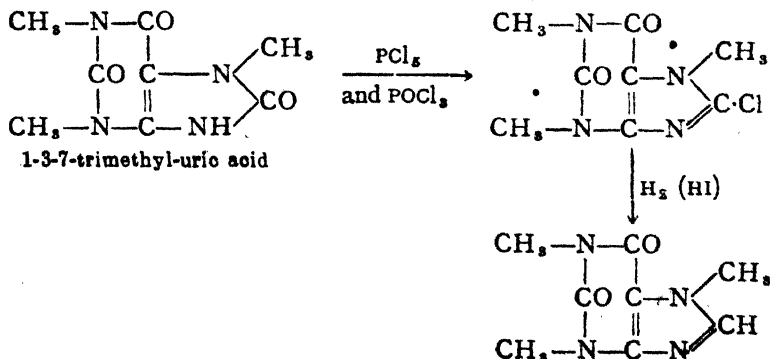
(enol form)
1-3-7-trimethyl-uric acid

Caffeine, therefore, must be represented by:



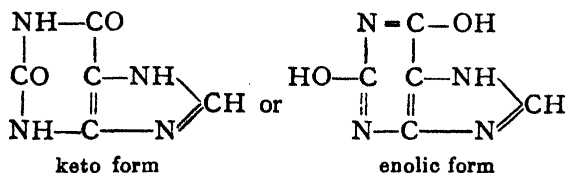
caffeine

Confirmation by Synthesis:—The above structure is finally confirmed by a simple synthesis from 1-3-7-tri-methyl-uric acid. The latter on treatment with a mixture of phosphorus oxychloride and phosphorus pentachloride, is changed into 8-chloro-caffeine which is readily reduced to caffeine. These changes can be followed schematically as follows:—



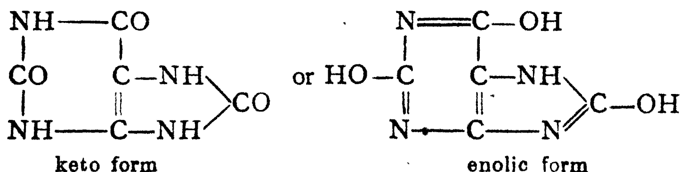
1-3-7-trimethyl-uric acid

STRUCTURE OF XANTHINE:—The structure of xanthine, the parent substance of the xanthine bases, follows from that of caffeine. Caffeine is proved to be trimethyl derivative of xanthine and hence xanthine must be:



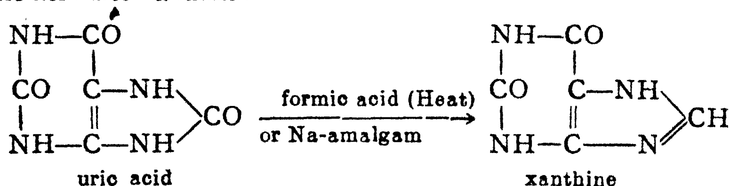
keto form or enolic form

But uric acid is:—



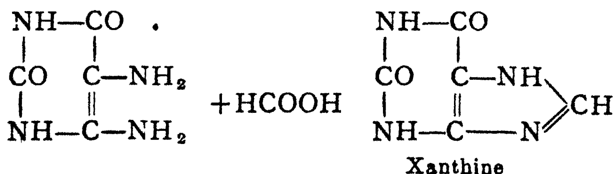
keto form or enolic form

Xanthine is, therefore, a reduction product of uric acid. This is further confirmed by the following straightforward transformation of uric acid into xanthine:—



uric acid — formic acid (Heat) or Na-amalgam → xanthine

The above structure for xanthine is confirmed by the Traube's synthesis. 4·5 diamino uracil obtained from cyano acetic ester and urea is condensed with formic acid, when xanthine is formed:



Xanthine

Xanthine is a crystalline compound sparingly soluble in water. It is weakly acidic and basic. Thus, hydrochloric acid gives $C_5H_4N_4O_2 \cdot HCl$.

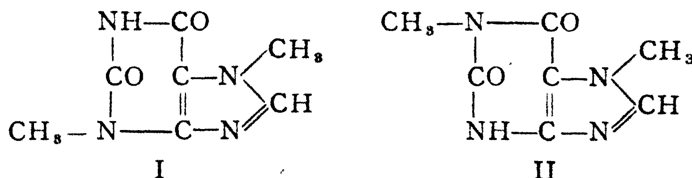
OTHER IMPORTANT XANTHINE BASES :—*Theobromnie and theophylline* are the two isomeric dimethyl-xanthines. Theobromine is found in the cocoa beans to the extent of 1·8 per cent. It is a crystalline white powder readily soluble in hot water. It finds use as a diuretic.

The constitution is established as follows :—The molecular composition is $C_7H_8N_4O_2$.

(*a*) Herzig determination shows the presence of two methyl groups.

(*b*) With Cl_2 water, theobromine gives mono-methyl alloxan and mono-methyl-urea. Hence the two methyl groups are distributed between the alloxan and the iminazole rings.

(*c*) Further methylation of theobromine (*i.e.* action of CH_3I on the Ag salt), gives caffeine which is 1, 3, 7, tri-methyl xanthine. Hence theobromine must be I or II.



But theobromine can be synthesised from (*i*) 7-methyl uric acid and (*ii*) 3-methyl uric acid, by methylation, subsequent reaction with PCl_5 & $POCl_3$ and reduction of the chloro compound. Now under the above experimental conditions :

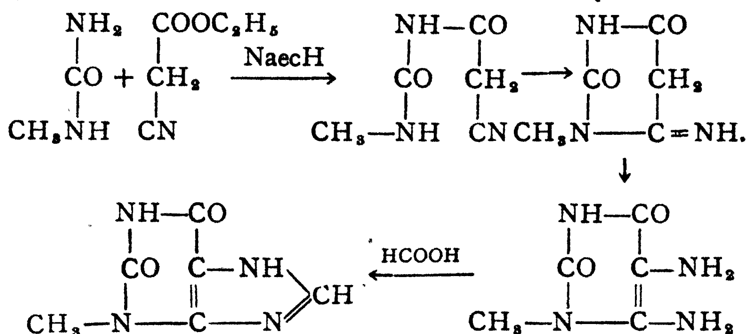
(*a*) 7-methyl uric acid can give rise to 3, 7 and 1, 7 dimethyl xanthines.

(*b*) 3-methyl uric acid can give rise to 3, 7 and 1, 3 dimethyl xanthines.

Therefore, theobromine must be 3, 7 dimethyl xanthine *i.e.* formula I must be assigned to it.

Theobromine can be synthesised by heating the lead salt of xanthine with CH_3I at 100°.

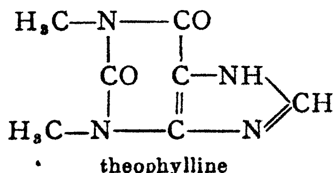
The above structure is established conclusively from its synthesis from 3 methyl xanthine. The latter is obtained from CH_3—NH—$CONH_2$ and cyano acetic ester by the Traube's method.



(the condensation between methyl urea and cyano acetic ester takes place as indicated above as the condensation product does not carry a free NH_2 group).

3 methyl xanthine is methylated to give theobromine which is soluble in alkali. Had methylation occurred at 1 position, the dimethyl derivative would be insoluble in alkali. Hence methylation takes place at position 7. This confirms the structure assigned.

Theophylline which is present in small quantities in tea leaves. is the 1-3-dimethyl-xanthine derivative; it is a crystalline powder m. p. 264°.



theophylline

Like theobromine, on methylation, it is changed into caffeine, It also finds use as a diuretic. It is more favoured than theobromine.

The exact position of the methyl groups in this xanthine base is established by a study of the products of oxidation with Cl_2 water; thus theophylline with Cl_2 water gives 1, 3 dimethyl alloxan and urea. These results thus prove that it is 1, 3-dimethyl xanthine. Theophylline is obtained in large quantities, starting from 1-3 dimethyl urea

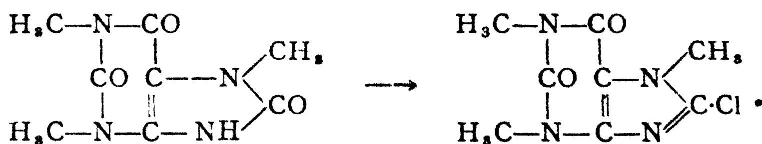
and cyano-acetic ester. 1, 3 dimethyl urea is obtained by the action of methylamine on urea at 140–160°.

Xanthine and its methyl derivatives, are basic because of the presence of the imino (*NH*) group in position 7, which is not adjacent to a carbonyl group. Hence, xanthine, theobromine and caffeine are referred to as xanthine bases and formerly they were classified as alkaloids.

General methods for the synthesis of xanthine bases :—There are two important methods developed by Fischer and by Traube for the syntheses of these bases. Almost all the xanthine bases have been obtained in this way.

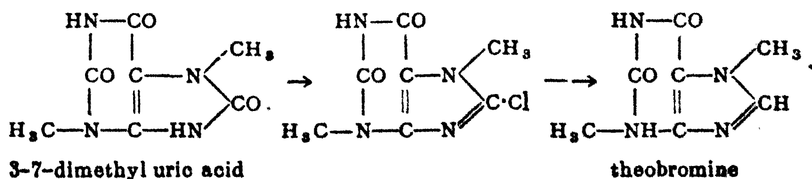
FISCHER'S SYNTHESIS :—The starting-point is uric acid or the methylated uric acid. On treatment with phosphorus oxychloride, uric acid or its methyl derivative is converted into the corresponding 8-chloro-purine derivative, which, on reduction, gives the corresponding xanthine base. Thus, we have :—

(*a*) *Caffeine synthesis* :—1-3-7-tri-methyl uric acid is changed into 1-3-7-tri-methyl-8-chloro-uric acid, by the action of phosphorus oxychloride :—



The latter, on reduction with hydriodic acid and red phosphorus at O° is converted into 1-3-7-tri-methyl-xanthine, *i.e.* caffeine.

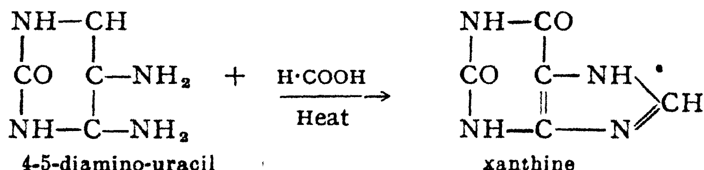
(*b*) *Theobromine synthesis* :—3-7-Dimethyl uric acid can be transformed into theobromine in an analogous way:



3, 7–Dimethyl uric acid is obtained from mono–methyl urea and malonic acid, and subsequent methylation with CH_2O and reduction with HI.

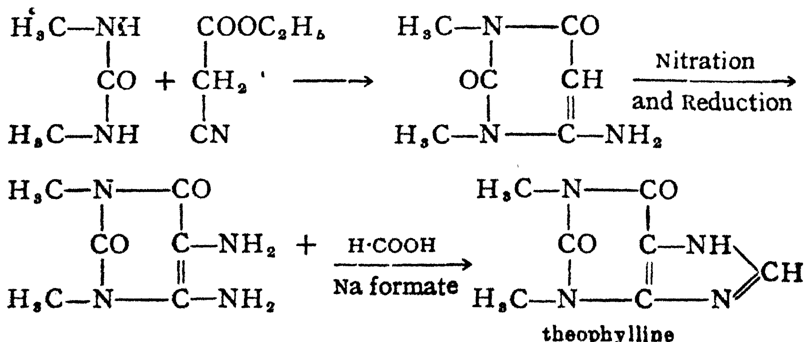
This method is very fruitful and all the xanthine bases are obtained in this way. The uric acid is first converted into the chloro-compound by the action of phosphorus pentachloride or phosphorus oxychloride at 140° and subsequently reduced with concentrated hydriodic acid. It is also noticed that the methylation of the chloro-compound is more easily effected than that of the reduced compound. Hence, the chloro-compound is first methylated and then reduced to the corresponding xanthine base by concentrated hydriodic acid. Recently Blitz has shown that uric acid or its derivatives can be directly reduced to xanthine system by heating with formic acid.

TRAUBE'S SYNTHESIS :—This is a simple and elegant synthesis of wide application. The starting–materials are urea and cyano-acetic acid or its ester. The cyano–acetyl urea first formed is converted into 4–5–diamino–uracil (*q.v.*). The diamino–uracil, on heating with formic acid, yields xanthine :—



4-5-diamino-uracil xanthine

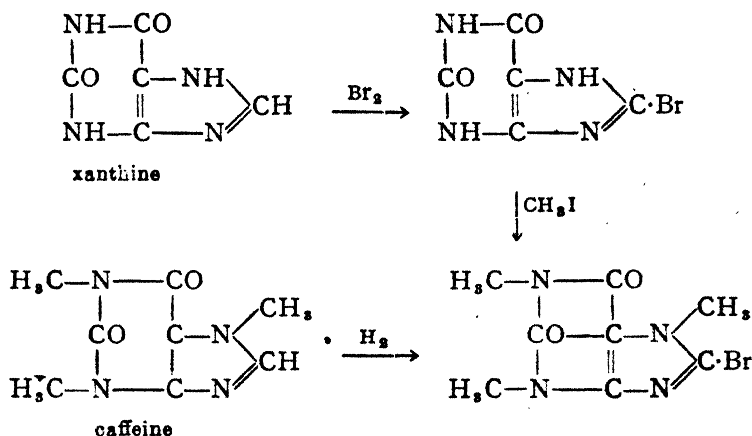
Starting with methylated ureas, the corresponding xanthine bases can be built up. Theophylline can, thus, be obtained from 1-3-di-methyl urea and cyano-acetic acid :—



theophylline

Theobromine can be similarly synthesised starting from mono methyl-urea and cyanacetic ester.

Caffeine is conveniently obtained from xanthine which is first synthesised :—



(The bromo-compound is much more readily methylated than xanthine itself).

The direct methylation of xanthine has been investigated by many chemists. As a result of these studies it has been found that: —

(1) Alkylation of xanthine or its salts with alkyl halides is difficult and hence, not practicable.

(2) Halogen derivatives of xanthine are, however, more readily alkylated.

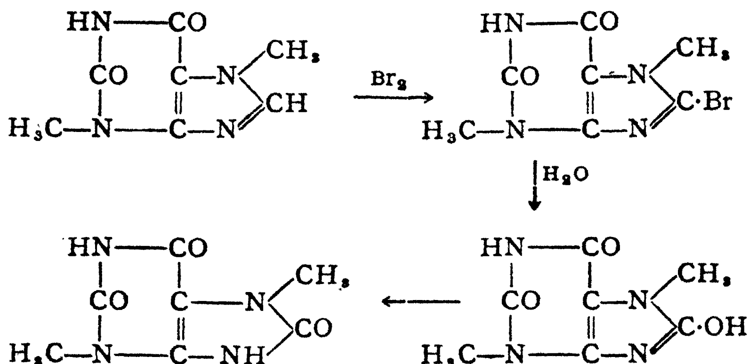
(3) The order of decreasing ease of the replaceability of the hydrogen atoms is 3, 7, 1.

(4) Dimethyl sulphate and alkali convert xanthine into caffeine very readily.

(5) The action of diazo-methane is very slow.

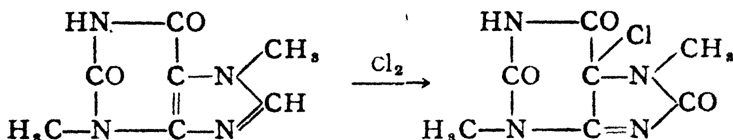
CONVERSION OF XANTHINE BASES INTO CORRESPONDING URIC ACIDS :—Fischer has effected the reverse change by converting the xanthine bases into their 8–halogeno-derivatives by the action of halogens like chlorine or bromine in an inert solvent like chloroform.

On hydrolysis with alkali, the halogen atom is replaced by hydroxyl group giving the corresponding uric acid derivative. Theobromine can, thus, be changed in 3·7 dimethyl uric acid :—



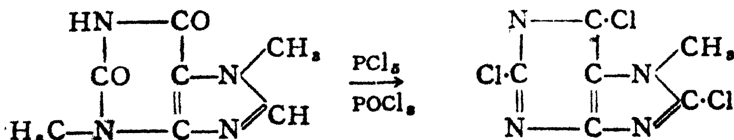
Recently, a new method of conversion has been reported by Blitz. It is of limited application only. Theobromine has been converted into 3-7-dimethyl uric acid in the following way :—

Theobromine is treated with chlorine in acetic acid solution to form 5-7 dimethyl-5-chloro-iso-uric acid:—

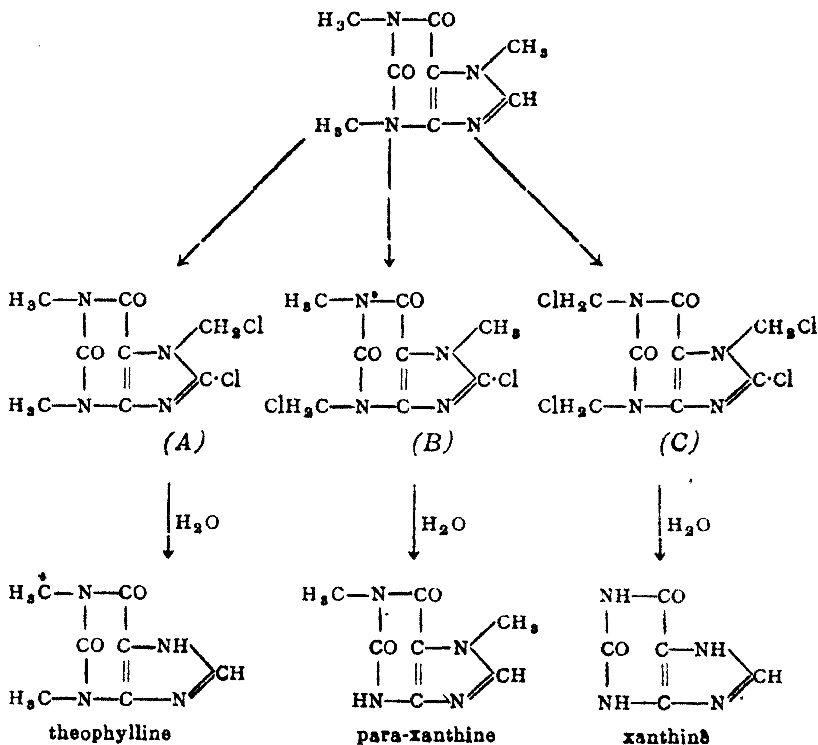


The latter, on reduction with $SnCl_2$, gives 3·7-dimethyl uric acid.

DEMETHYLATION OF XANTHINE BASES :—The methylated xanthine, when heated with a mixture of the phosphorus halides at 140° to 160°, suffer demethylation, and substitution in positions 2, 6 and 8 takes place. Theobromine, thus, gives :—



Caffeine can be demethylated to give theophylline, paraxanthine and xanthine. On heating with phosphorus pentachloride or phosphorus oxychloride, containing chlorine, caffeine gives the chloro-compounds *(A)*, *(B)*, *(C)*, which, on hydrolysis and subsequent reduction, with HI at room temperature gives the corresponding demethylated xanthine :—



The demethylation of caffeine takes the following course :—

(1) The hydrogen atom in position 8 is first replaced by chlorine atom.

(2) Chlorine atom then enters the methyl groups successively in positions, 3, 7 and 1.

(3) The CH_2Cl group is eliminated by heating with water :—

$$>N{-}CH_2Cl + CH_2O \longrightarrow >NH + CH_2OHCl \longrightarrow CH_2O + HCl$$

(4) The xanthine type is finally obtained by reduction *i.e.*, the replacement of the chlorine atom in position 8 by hydrogen.

Amino-purines :—Adenine and guanine are the two purines which contain NH_2 groups. They are the products of hydrolysis of nucleic acids and are found widely distributed in plants and animal organisms.

ADENINE :—It is found in tea leaves and molasses and in large quantities in yeast. Its molecular composition is $C_5H_5N_5$. Its structure is based on the following reactions :—

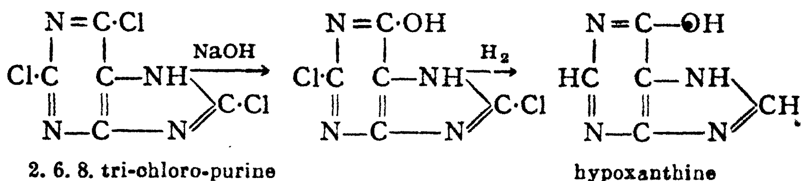
(*a*) with nitrous acid, adenine is converted into hypoxanthine ;



$$C_5H_5N_5 + HNO_2 \rightarrow C_5H_4N_4O + N_2 + H_2O$$

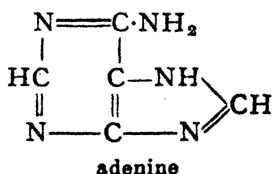
This indicates that adenine contains an NH_2 group.

Now, hypoxanthine is obtained from 2-6-8-tri-chloro-purine by the action of alkali and subsequent reduction with hydriodic acid. Hence, it has been assigned the structure (*A*) :—



2. 6. 8. tri-chloro-purine hypoxanthine

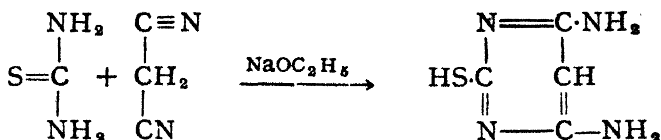
Adenine is the corresponding amino derivative and is therefore to be represented by :—



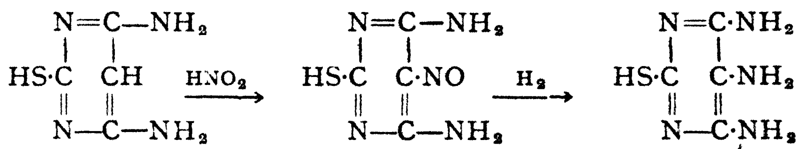
adenine

(*b*) Adenine has been synthesised by Traube by a straightforward and unambiguous method which leaves no doubt regardnig its constitution. The essential steps in the synthesis are :—

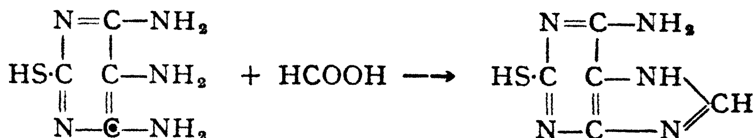
Thio-urea and the nitrile of cyano-acetic acid are condensed in presence of sodium ethoxide :—



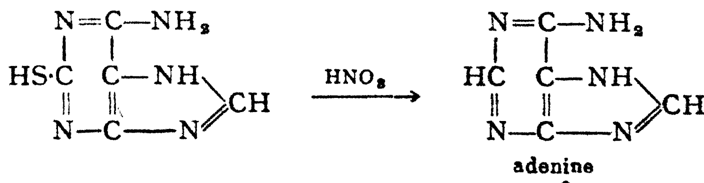
The 4-6 diamino compound obtained, is then treated with nitrous acid to the 5-nitroso-derivative which, on subsequent reduction, gives the 4-5-6-tri-amino-derivative :—



The tri-amino-derivative is next condensed with formic acid :—

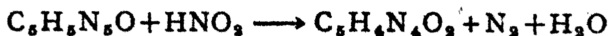


The thio-derivative, on oxidation with dilute nitric acid, is converted into adenine :—



GUANINE :—It is found in the scales of fish and reptiles to which it gives the characteristic shiny surface. It is also present in guano. It is used in the preparation of artificial pearls. The constitutional formula of guanine is established both by analytical and synthetical evidence.

(*a*) With nitrous acid, guanine is converted into xanthine.

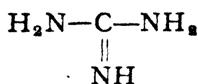


$$C_5H_5N_5O + HNO_2 \longrightarrow C_5H_4N_4O_2 + N_2 + H_2O$$

This indicates that guanine differs from xanthine in the possession of an amino group in place of one of its hydroxyl groups. Now xanthine carries hydroxyl groups in position 2 and 6. Hence, the amino group in guanine must be in one of these positions (2 or 6).

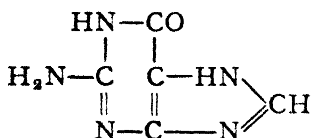
The position of the amino group is then fixed by the following evidence :—

(*b*) On oxidation, guanidine is formed, which has the structure :—



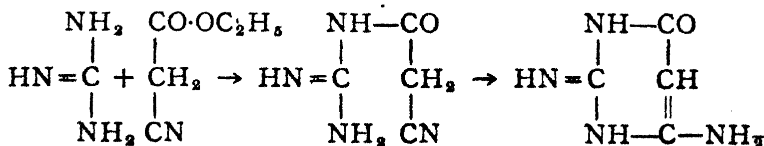
The amino group (NH_2) is, therefore, in one of the urea residues in the molecule, *i.e.* it must be present in position 2 or 8.

From (*a*) and (*b*) it, therefore, follows that the NH_2 group must be present in position 2 and that guanine must be assigned the constitution :—



The above structure has been confirmed by a synthesis. (Traube). The steps involved are :—

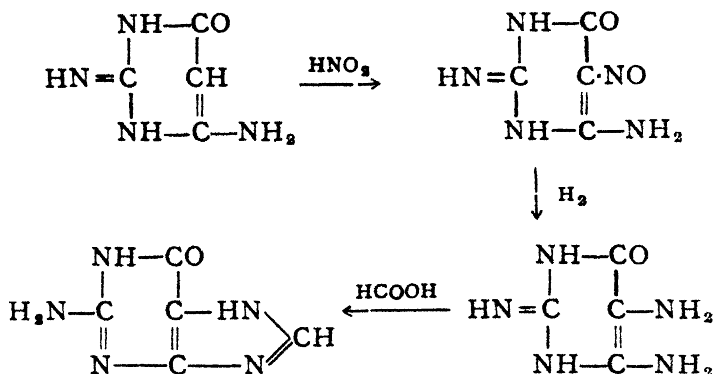
Guanidine is condensed with cyano-acetic ester in presence of sodium ethoxide.



Cyclisation and isomerisation occur at the same time.

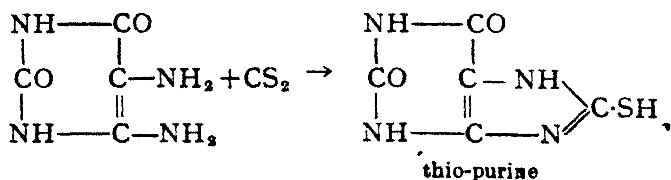
The corresponding 4-5-di-amino derivative is then obtained by the action of nitrous acid and subsequent reduction of the nitro

compound. (*Cf.* Traube's synthesis of uric acid and xanthine bases.) The diamino compound, is then condensed with formic acid to give guanine.

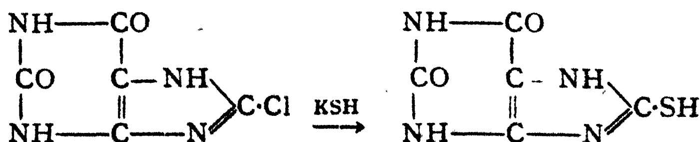


Thio-purines :—They were first obtained by Fischer from the halogenated purines by the action of carbon disulphide or metallic hydro-sulphides. The 8-thio-derivatives are the most interesting as they can be readily oxidised to the corresponding xanthine derivatives. Recently, a number of methods have been developed for their synthesis.

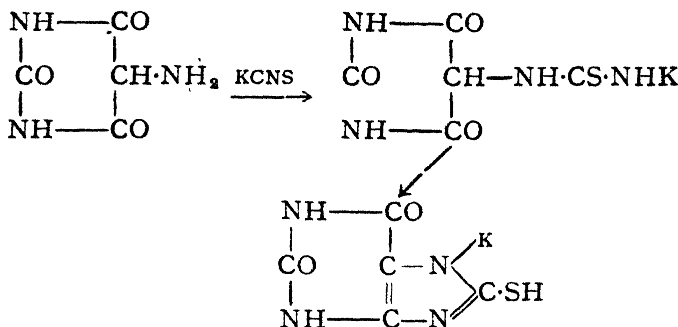
(1) From 4-5-di-amino-uracil and carbon disulphide :—



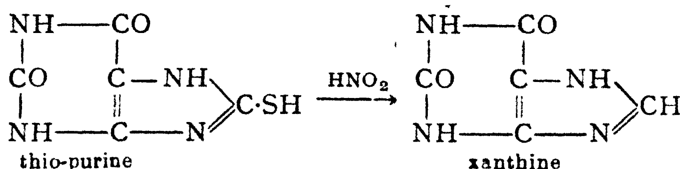
(2) From 8-halogeno purines and potassium hydrogen sulphide :—



(3) From uramil and iso-thiocyanate :—

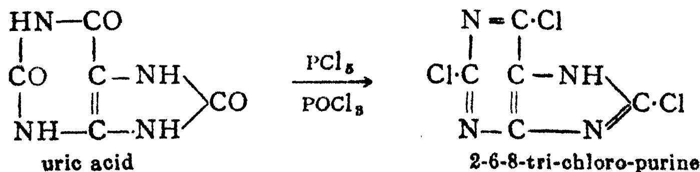


These thio-purines, on treatment with nitrous acid, are converted into xanthines :—

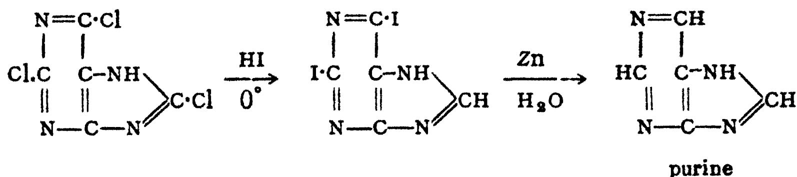


We shall now discuss the parent substance from which uric acid and all the xanthine bases are derived. It is obvious from the structural relationships of these compounds that they all possess the same common fundamental unit. Fischer envisaged a hypothetical compound that would result from the reduction of uric acid till it contained only the elements carbon, hydrogen and nitrogen, without affecting the ring structure. He called it '*purine*' but he had to wait for fourteen years before he could actually isolate it.

Purine :—It does not occur in nature. It has been synthesised by Fischer from uric acid in the following way ;—Uric acid is heated with an excess of phosphorus oxychloride and PCl_5 at higher temperatures (160–170°) when chlorine atoms substitute successively in positions 2, 6 and 8, and tri-chloro-purine is obtained :—

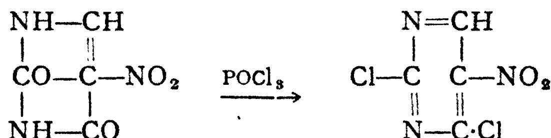


The tri-chloro-compound was then reduced with concentrated hydriodic acid at 0° to di-iodo purine and finally, to purine by the action of zinc dust and water.

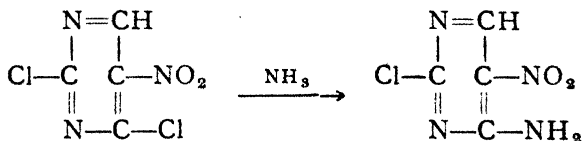


Another complete synthesis of purine, starting from 5-nitro-uracil has been achieved by Isay. The steps involved are:—

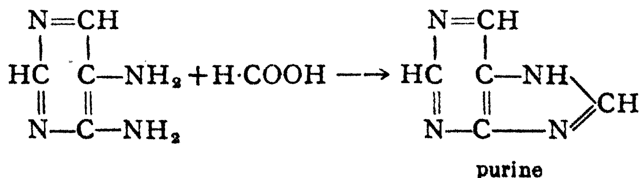
5-Nitro-uracil is heated with phosphorus oxychloride to give 2-4-dichloro-5-nitro-pyrimidine :—



On treatment with ammonia, 2-chloro-5-nitro-4-amino-pyridine is obtained :—



Reduction of the latter gives 4-5-diamino-pyrimidine, which on condensation with formic acid at 210°, yields purine :—



Purine is a crystalline compound, soluble in water; it melts at 216°. It gives salts with acids and alkalies.

Relation of uric acid and xanthine bases to purine :—The molecular compositions of purine, xanthine, uric acid, etc. are :—

Purine	— $C_5H_4N_4$
Hypoxanthine	— $C_5H_4N_4O$
Xanthine	— $C_5H_4N_4O_2$
Uric acid	— $C_5H_4N_4O_3$
Adenine	— $C_5H_5N_5$

from which it follows that :—

(*a*) Hypoxanthine is mono-oxy-purine

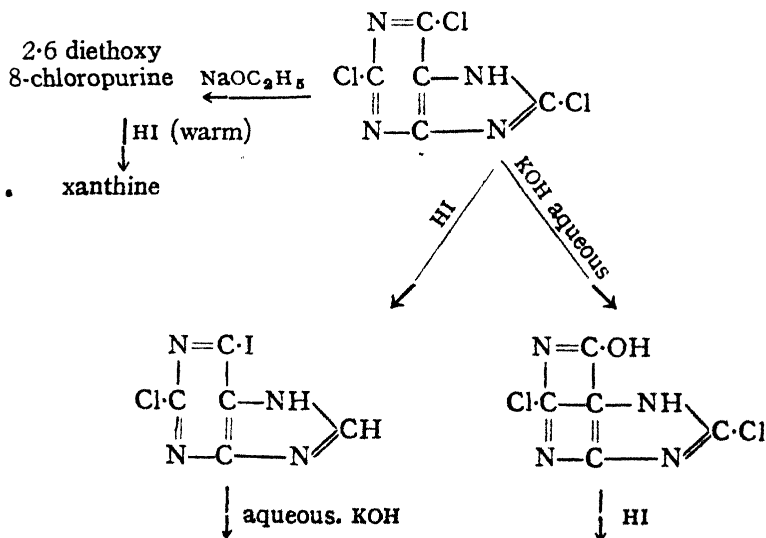
(*b*) Xanthine is di-oxy-purine

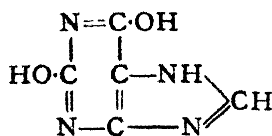
(*c*) Uric acid is tri-oxy-purine.

(*d*) Adenine is mono-aminc-purine

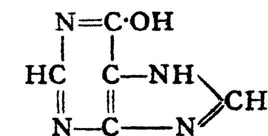
These relationships have been actually experimentally verified. Thus, starting with tri-chloro-purine, Fischer synthesised purine, xanthine, hypoxanthine, adenine etc.

(a) Syntheses of oxy-purines :—





2-6-dioxy-purine
(xanthine)

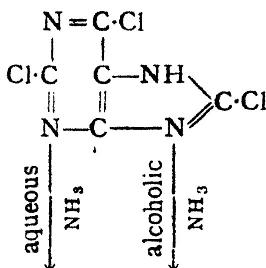


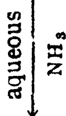
6-oxy-purine
(hypoxanthine)



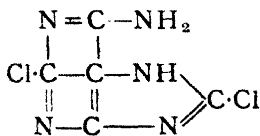
Theobromine → Caffeine

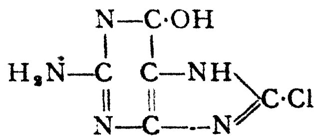
(b) Syntheses of amino-purines :—

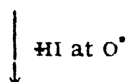




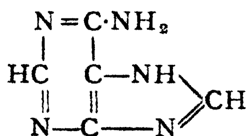




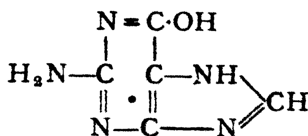








adenine



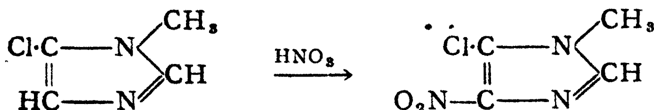
guanine

The above conversions of tri-chloro-purine into the different purine bases have served to establish the structural formulas of these compounds and also their internal relationships. Secondly, as the chlorine atoms in tri-chloro-purine are very reactive and capable of being replaced by *OH*, *SH*, NH_2, *I*, etc. it is possible to obtain a large number of derivatives in addition to those that occur in nature.

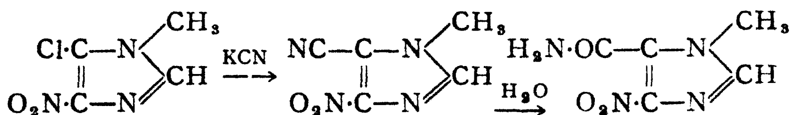
Saransin and Wegmann's Synthesis of Purines :—The starting-point is an imidazole derivative. The synthesis of hetero-xanthine (7-methyl-xanthine) has been accomplished as follows :—

Dimethyl-oxamide is treated with phosphorus pentachloride to from 1-methyl-5-chloro-imidazole.

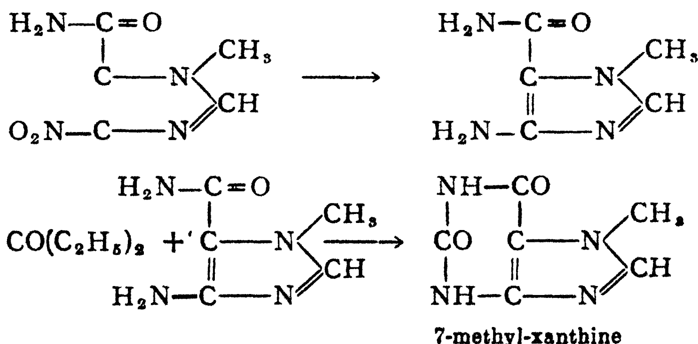
With nitric acid the 4-nitro derivative is obtained :—



The chlorine atom is then replaced by *CN* group by the action of potassium cyanide. The nitrile, so formed, is partially hydrolysed to the corresponding amide ($CN \rightarrow CO \cdot NH_2$) :—

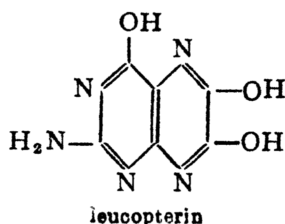


The nitro group is reduced to amino and the diamino compound is condensed with ethyl carbonate when hetero-xanthine is formed.

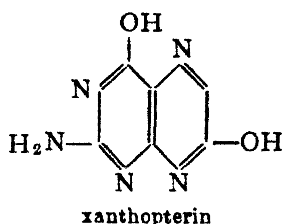


Purine derivatives as Natural Colouring Materials:— The colouring materials from the butterfly wings have been shown to be purine derivatives, and are referred to as pterines by Wieland. The yellow pigment is called xanthopterin and the white pigment is known as leucopterin. The latter is synthesised by condensing

2, 5, 6 tri-amino-4-hydroxy-pyrimidine with oxalic acid at 140°. Xanthopterin readily takes up an oxygen atom and is converted into leucopterin. Hence the structures for these pigments are :—



leucopterin



xanthopterin

Xanthopterin occours in mammalian tissues and probably plays an important rôle in oxidation—reduction systems present in living tissues.

CHAPTER X

SYNTHETIC DRUGS

Introduction :—The early history of chemistry is closely linked with the history of medicine, for, the practice of chemistry was exclusively in the hands of medical men. Paracelsus (1493-1541) who freed chemistry from the shackles of alchemical bounds insisted on making her a hand-maid of medicine. However, up to the 19th century, it was believed that the number of therapeutic substances comprised a limited number of substances extracted from parts of animals or from earth in the form of minerals. A strange mixture of superstition and science ruled their administration and complete ignorance prevailed regarding their action. It was but natural that in such an atmosphere, it was impossible to entertain an idea of creating entirely new remedies for combating the human diseases. The era of synthetic drugs had to await not only an advance in the technique of synthetic organic chemistry but also a full knowledge of physiology of human organism which was to act as a test tube in which these foreign substances were made to react.

A few principles have governed the synthesis of medicinals; they are based on the correlation of physical and chemical properties of a compound with its physiological action. An insight into such a correlation, has been obtained by three different methods of approach.

In the first method, the structure of a natural product with physiological activity, has been purposely altered. New groups are introduced or old ones are removed from the natural product, by chemical methods, and the compounds thus obtained are tested for their physiological activity. The antimalarials, pamaquinine, mepacrine and many others have been developed in this manner. The patient variations of structures, conditions, testing etc., have become the routine technique, which has rendered possible, the modern development of sulpha drugs, antihistamines and antitubercular agents.

The second method is to study the metabolic fate of a known drug, in a living animal. Under these conditions, the administered

compound is detoxicated and the chemical changes involved are: (*i*) oxidation, (*ii*) reduction, or (*iii*) conjugation with other compounds. A knowledge of these changes, allows important conclusion to be drawn regarding the effect of structural factors, on physiological activity. Thus the synthesis of many of the antipyretics derived from aniline have been the result of the observation of changes suffered by aniline and its derivatives in their passage through the system. Similarly, the observation that arsenic in atoxyl is converted into trivalent condition in the human body in order to be effective, led partly to the development and use of *salvarsan* in which arsenic is trivalent, as an important drug.

Lastly, the vast development of modern chemotherapy—founded by Ehrlich—had its roots in the theories of mechanism of action of the drug. Ehrlich's "dyestuff theory" is based on the observation that parasites can be stained selectively with synthetic dyestuffs; the parasites are thus fixed to their detriment, while the living tissue cells are not at all attacked. The substance is thus only parasito-tropic and not organo-tropic. This theory laid the foundation of modern chemotherapy and is responsible for the development of thousands of chemotherapheutic agents.

The more recent metabolite-antagonist theory of drug action, developed by Woods and Fildes, in connection with the mode of action of the sulpha drugs, has been very fruitful and has led to the synthesis of several hundreds of chemical compounds, which have been tested for their antibacterial, antihistaminic and antitubercular activity. The theory was originally proposed as an explanation of the bacteriostatic action of sulphanilamide. The latter is found to antagonise the action of para-amino benzoic acid which is the essential metabolite of many bacteria. The theory has been successfully extended to other cases, and it is now claimed that a fundamental explanation of the mode of action of a drug has been at last found in this theory.

Lastly, the concept of isosterism has been invoked to account for the observed similarity in physiological action of compounds which are structurally analogous. This has further led to the development of new compounds with actions similar of those of model drugs. Thus — N = is equivalent to — CH = and benzene and

pyridine are isosters; hence attempts have been made to replace benzene nucleus in a modern drug, by pyridine in the development of new drugs.

Constitution and Physiological Action

A close study of many natural drugs has revealed that the drugs owe their activity to the presence of certain parts with a specific structural pattern, in their molecules. Such a part of the drug molecule which is responsible for the observed physiological activity, is called a *pharmacophore* group. Thus the grouping



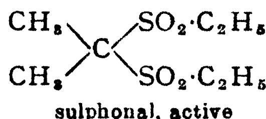
in the cocaine molecule (p. 387) is responsible for the local anæsthetic action; it is the pharmacophore. It is possible to obtain several compounds of similar activity, by varying the remainder of the molecule, but keeping the pharmacophore group intact. This principle has led to the attempts to improve known drugs. Physiological action of a chemical compound may also be profoundly altered by the introduction or suppression of an element or a group; at times it is possible to foretell this alteration by the study of the nature of the group that brings about the alteration. The group may exercise its own specific physiological action or may act as an anchoring group and thus, bring into play the action of the other part of the molecule to which it is attached. The main types of groups with their effect on the physiological action of a compound, are discussed below.

EFFECT OF ALKYL GROUPS:—It has been observed by Schsneideberg that the toxic effect of certain compounds is lessened by the introduction of alkyl groups. In support of this observation, it may be pointed out that the substitution of alkyl group for hydrogen in hydrogen cyanide *(HCN)* to give nitriles *(RCN)* or isonitriles *(RNC)* leads to less poisonous compounds. The convulsive properties possessed both by ammonia and aniline are diminished by the replacement of hydrogen attached to nitrogen while, on the other hand, it may be observed that xanthine, has no toxic action on the heart but its N-dimethyl and N-trimethyl derivatives theobromine and caffeine are known to exert powerful stimulating action on the heart. Further, alkylation is found to increase the toxicity; toluene

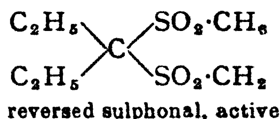
is more toxic than benzene; alkyl amines are more toxic than ammonia, and ethers are more toxic than alcohols. Thus methylation increases the toxicity of the resorcinol molecule. On the other hand, the poisonous nature of catechol is decreased by the methylation of the OH groups.

Lastly, there are certain observations which defy explanation. Dulcin which has the structure: *p* $C_2H_5O—C_6H_4—NH—CO—NH_2$, is 200 times as sweet as sugar, but the corresponding methyl derivative: *p* $CH_3O—C_6H_4—NH—CO—NH_2$ is tasteless. Similarly, it is very difficult to explain the loss in activity of the drug suramine, by the removal of the two methyl groups from the molecule.

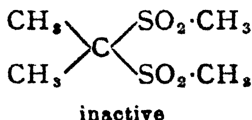
The comparison between the methyl and ethyl groups regarding their activity, shows that the latter has a hypnotic action which the methyl group lacks; sulphonal and even reversed sulphonal have the physiological effect of inducing sleep but if all ethyl groups are substituted by methyl groups, the compound formed is quite inactive.



sulphonal, active



reversed sulphonal, active



inactive

The experimental observations of Eherlich and Michael that certain dyes containing $N(C_2H_5)_2$ groups possess the property of dyeing the nerve fibres, which is lost on substitution of C_2H_5 by CH_3, shows that C_2H_5 group must be acting as an anchoring group.

EFFECT OF HYDROXYL GROUP :—It is surprising to find that the effect of hydroxyl group is different and distinct in the aliphatic and the aromatic compounds; in the case of the first, it generally brings about a weakening of the physiological action and in the latter, it intensifies the action and confers a strong antiseptic property. For example, if the aliphatic alcohols are narcotic and poisonous, increase in the hydroxyl groups in the molecule as in the glycols and

glycerol makes them inactive ; aldehydes exercise narcotic action, but their conversion into aldols by introduction of hydroxyl group renders them inactive, while more *OH* groups as in glucose, makes the compound sweet in taste and as an essential food.

The entrance of hydroxyl groups in the aromatic compounds makes the new compound more toxic. Phenol has more toxicity and strong antiseptic properties than benzene, while introduction of hydroxyl group in the benzoic acid to give salicylic acid confers on the compound, the antiseptic property as well as specific action against rheumatism. Polyphenols are also more toxic than phenol. Secondary and primary alcohols have decreasing order of activity, while in homologous series, members with long sidechains are more active.

EFFECT OF ALDEHYDIC AND KETONIC GROUPS :—No broad generalisation is possible; the aldehyde group is present in such divergent types of compounds as chlorophyll, vanillin and streptomycin. Formaldehyde has a strong irritating action on the mucous membrane but is also a powerful antiseptic and has a hardening effect on the tissues for which property it is used by taxidermist. The higher homologues possess the property of the aldehydic groups combined with the properties of the alkyl radical. However, the entrance of hydroxyl groups in the aldehyde molecule brings about a lowering of the activity, which as in the case of sugars may completely disappear.

Ketonic group confers on the molecule narcotic properties. The aliphatic ketones, show this property in a very marked degree and acetophenone ($C_6H_5COCH_3$) which is a mixed ketone is used as a narcotic under the name of hypnone.

EFFECT OF ACID GROUPS :—The diminution in the physiological effect is the outcome of the entry of an acid group in the molecule. Poisonous phenol (C_6H_5OH) is rendered harmless in phenyl sulphuric acid $C_6H_5OSO_2OH$. Similarly, the amines are toxic while the amino acids are foods. It is not necessary that the entry of a group should be accompanied with the disappearance of *OH* group but even an introduction of an acid group anywhere in the nucleus makes it less harmful. Thus nitrobenzene is poisonous, while the nitro-benzoic acids are harmless. However, if the acid is

esterified, the original physiological properties re-appear; tyrosine is not poisonous, but its ethyl ester possesses poisonous properties. The observed effect of the acid groups (COOH and $—SO_3H$) in suppressing toxicity, is taken advantage of in the preparation of many synthetic drugs. A compound retains its activity on acetylation or benzoylation; because after hydrolysis in the body, it becomes free to exert its original physiological action. It is also very significant that in the body, detoxication of a poisonous compound is effected by conjugation with glycine $H_2N·CH_2—COOH$, which is an amino-acid.

EFFECT OF HALOGENS :—The entrance of *Cl* in the molecule of aliphatic compounds and aromatic compounds gives rise to different results. While in the aromatic series, the change in the physiological properties is very slight in the aliphatic compounds, substitution brings about an increase in the narcotic action but is accompanied with depressant action on heart and blood vessels. This is very well brought out in the series: methyl chloride (CH_3Cl), methylene chloride (CH_2Cl_2) chloroform ($CHCl_3$) and carbon tetrachloride (CCl_4) wherein an increase in narcotic action and toxicity increases with the atoms of *Cl* present in the molecule.

It is also observed that halogens increase both useful and toxic properties of active compounds, but not at the same rate. Hence halogen substitution is resorted to, as a means of stepping up activity as also of widening the margin of safety in a given series. With the halogens themselves, *Cl*, *Br* and I, the hypnotic properties decrease with increasing atomic weight but the antiseptic properties increase as is shown with chloroform, bromoform and iodoform. The toxicity of iodine compounds is more than that of analogous chlorine and bromine compounds but they function as effective antiseptics and hence many iodine compounds are used for these purposes. Fluorinated compounds are much less physiologically active than the corresponding non-fluorinated compounds. This is ascribed to the great stability of the fluorine derivatives.

EFFECT OF NO_2 AND *NO* GROUPS :—The entrance of a nitro group into aromatic compounds, increases the toxicity; this is shown by the fact that nitro-benzene and nitro-naphthols are more poisonous than the substances from which they have been derived.

However, if in addition to the nitro group, there is another substituent like CH_3 or CHO, in the nucleus, which can be oxidised in the body to the $COOH$ group, the toxicity decreases; this is borne out in the case of the compound *para*-nitro-toluene and aromatic nitro-aldehydes.

Aliphatic nitro-compounds (RNO_2) which are isomeric with the nitrites ($R-ON=O$) differ from them in their action; for the nitrites have the property of dilating the blood vessels, while nitro compounds have no such physiological action.

EFFECT OF NH_2 GROUP:—The amino group is toxic, but successive alkylation reduces the toxicity. Acylation generally decreases the physiological activity. Aniline is thus highly toxic, but acetanilide is used as a febrifuge. Sulphonation and carboxylation of aromatic amines also causes a marked decrease in the physiological activity. Polyamines are more toxic than the mono amines; the phenylene-diamines are very poisonous.

However, many antipyretics and analgesics are known, which are derivatives of aromatic amines. The aromatic derivatives of hydrazine also possess antipyretic properties; antipyrine and pyramidon belong to this group.

EFFECT OF CYANOGEN GROUP:—The introduction of the cyanogen group, may give rise to two distinct series of compounds, the nitriles $RC\equiv N$ and the iso-nitriles $RN=C$ and both these types possess, though to a less degree, the poisonous nature of the parent substance hydrocyanic acid, HCN. If the iso-cyanides bring about paralysis of the respiratory system, the nitriles cause comatic condition. In the case of nitriles of aliphatic series, the lower members are more poisonous than the higher ones.

EFFECT OF UNSATURATED LINKAGES:—Unsaturation introduces in the compound, a centre of chemical activity and hence unsaturated compounds possess far more toxic properties than the corresponding saturated ones. Unsaturated alcohols, as against the saturated ones, possess distinct poisonous properties, Propyl alcohol, which possesses only the usual physiological properties of alcohols, in being an intoxicant is not poisonous, while the unsaturated allyl alcohol, $CH_2=CH-CH_2-OH$ as strong

poisonous properties. The toxicity of a compound increases with increasing unsaturation.

EFFECT OF ISOMERISM :—(*a*) Structural isomers often show marked difference in physiological activity. Thus α-cocaine which differs from cocaine, in the position of the—$COOCH_3$ group, has no local anæsthetic action at all. Similarly, the *ortho*, *meta* and *para* derivatives in the aromatic series exhibit great differences in their physiological activities. In the case of mono halogenated phenols, the meta isomers are the most active as antiseptics. Similarly sulphanilamide is very active as a drug, while the corresponding isomers are inactive.

(*b*) Stereo-isomers both geometrical and optical, possess different physiological properties. If maleic acid is poisonous to dogs, the isomeric fumanic acid is harmless. Similarly, *l*-nicotine is twice as poisonous as the *d*-form : and *l*-adrenaline is twelve times as active as the *d*-isomer.

Classification of the drugs :—The drugs may be classified according to either the chemical type—the structural unit—or the nature of their therapeutic action. But a rigid classification, based on chemical structure, would split up groups of drugs which have the same physiological action, while a therapeutical classification will tend to obscure the chemical resemblances and thus render a description of the chemistry of the different types of drugs, extremely difficult. A compromise is to group them first on the basis of their therapeutic action *e. g.* hypnotics, antiseptics, antipyretics etc. and then subdivide the group according to the chemical structure of the drug. In a few cases, it is more convenient and conducive to study, to group the drugs arbitrarily ; the synthetic hormones with different chemical structure and different physiological action are thus grouped together ; and similar is the case with the synthetic vitamins. In what follows, the discussion of the drugs has been undertaken on the basis of the compromise classification. Thus we have discussed the following types of drugs :

1. Antipyretics and analgesics
2. Local anesthetics
3. Narcotics : hypnotics, sedatives and anesthetics

4. Antiseptics
5. Chemotherapeutic agents :
   (*a*) Antibacterials
   (*b*) Antimalarials
   (*c*) Antihistamines
   (*d*) Trypanocides
   (*e*) Sulphadrugs
   (*f*) Anti-biotics.
   (*g*) Amoebicides
   (*h*) Sympathomimetic drugs
   (*i*) Anthelmintics
   (*k*) Anti-tubercular drugs
6. Synthetic vitamins
7. Synthetic hormones
8. Miscellaneous drugs :
   (*a*) Cardiac stimulants (cardiovascular drugs)
   (*b*) Diuretics
   (*c*) Purgatives
   (*d*) Mydriatics
   (*e*) Anticonvulsants
   (*f*) Diagnostic reagents.
   (*g*) Muscular relaxasts (hypo-tensive drugs)

Antipyretics—Analgesics

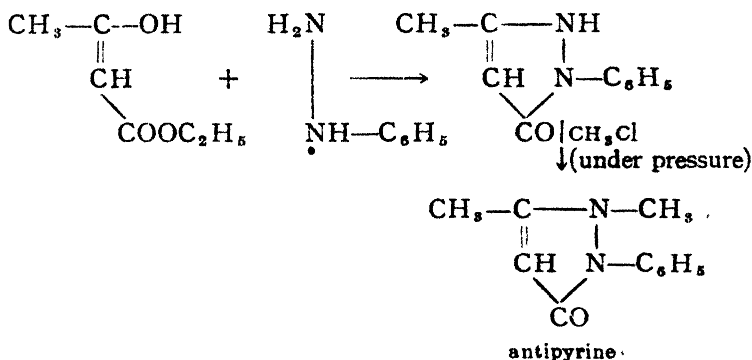
Prior to the introduction of synthetic antipyretics, quinine was known as a valuable antipyretic with additional advantage of having specific action against malaria and other intermittent fevers. The quinine molecule contains a quinoline ring, and it was but natural to recognise in, this nucleus, its antipyretic action and attempts were made to prepare compounds which were quinoline derivatives and which possessed antipyretic properties. Thalline, kairoline and kairine were the direct result of such attempts. They are all quinoline derivatives, possessing valuable antipyretic properties ; but they are no longer used in medicine because of their highly toxic side effects.

Antipyretics usually tend to produce sleep and cause relief of some kinds of physical pain, and thus act as analgesics *i. e.* pain-relievers. The most commonly used antipyretics—analgesics fall into the following fundamental structural types :

(*i*) Pyrazolone derivatives
(*ii*) Salicylic acid and salicylates
(*iii*) Derivatives of aniline and of para-amino phenol
(*iv*) Quinoline derivatives.

PYRAZOLONE DERIVATIVES: The first important and useful synthetic antipyretic, ***antipyrine***, was prepared by Knorr in 1884. At first, he assigned a quinoline structure to the new antipyretic, but subsequently it was shown to be a pyrazolone derivative. This discovery was followed by the preparation of many pyrazolone derivatives; the most successful of them are antipyrine and aminopyrine or pyramidon.

ANTIPYRINE: It is obtained by condensing ethylaceto acetate with phenyl hydrazine at 120° and subsequently methylating the resulting methyl-phenyl-pyrazolone.



antipyrine

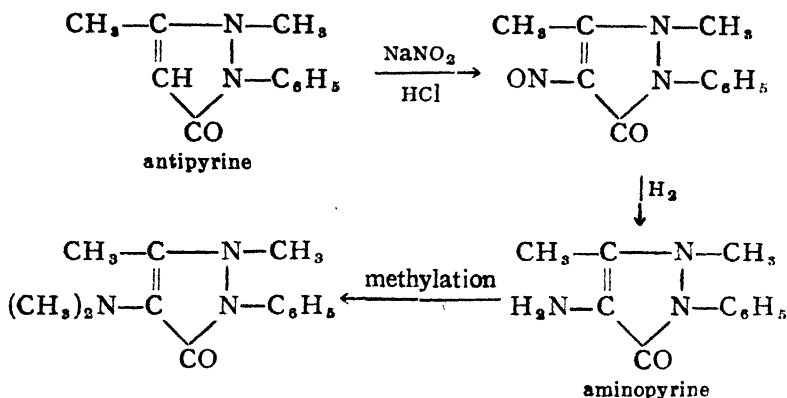
Alternately, antipyrine can be directly obtained by condensing N-methyl-phenyl hydrazine with ethyl acetate.

Antipyrine is a colourless, odourless, crystalline, white powder with slightly bitter taste. Its antipyretic action is even greater than that of quinine but unlike it, it has no action against malaria. It has also the property of diminishing body pains but has slight action on the heart.

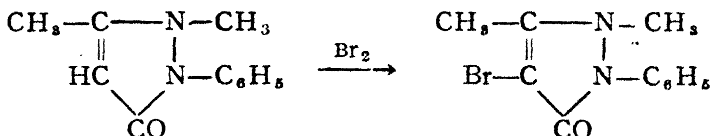
AMINO-PYRINE OR PYRAMIDON:—Of the many derivatives of antipyrine which are used to serve the same purpose as the parent body, without its injurious effect on the heart, the most important is pyramidon. It is the $N(CH_3)_2$ derivative which is much more powerful than antipyrine itself.

A solution of antipyrine in dilute hydrochloric acid is treated with sodium nitrite resulting in the nitroso compound. The latter is reduced to amino-antipyrine with acetic acid and *Zn* dust. The

amino-antipyrine is separated in a crystalline form by treatment with benzaldehyde forming a benzylidene derivative. This is then decomposed with dilute HCl and the amino compound subsequently methylated. Methylation is effected by treating with chloro-acetic acid and subsequent decarboxylation of the diacetic acid derivative obtained, by heating it in an autoclave with HCl at 120°.



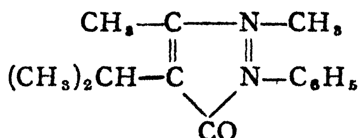
In another method, 4-bromo antipyrine is first obtained:



which is then condensed with $NH(CH_3)_2$ to give the drug.

Pyramidon is a yellowish-white powder which is soluble in alcohol, ether and benzene. It is like antipyrine, both analgesic and antipyretic though nearly three times powerful than antipyrine and therefore, is given in small doses.

Erlenmeyer and Wile have obtained a new antipyretic which contains an isopropyl group in place of dimethyl amino group in amino pyrine. This is based on the principle of isosterism. The drug has the structure:



It possesses very valuable antipyretic properties.

(*ii*) DERIVATIVES OF ANILINE:—Aniline itself possesses antipyretic action but is accompanied with bad after-effects. Hence a few derivatives are used. Some of the most important of these are acetanilide and phenacetin.

ACETANILIDE ($C_6H_5 \cdot NH \cdot COCH_3$) Antifebrin, is prepared by heating a mixture of equal weights for pure aniline and glacial acetic acid in a steam jacketed still, at a temperature of 120° to 125° with $ZnCl_2$ as a condensing agent. Acetanilide separates out, when the mixture after completion of the reaction, is poured in cold water:

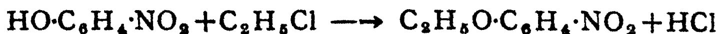


$$C_6H_5NH_2 + HOOC{-}CH_3 \longrightarrow C_6H_5 \cdot NH \cdot COCH_3 + H_2O$$

It forms colourless glistening crystals (m.p. 115°C); it dissolves in hot water and in alcohol. In addition to possessing antipyretic action, it has also anti-neuralgic property but is not entirely free from harmful effects of aniline as the acetyl group is oxidised in its passage through the body. Methyl acetanilide $C_6H_5N(CH_3)CO \cdot CH_3$, is also used as a febrifuge under the name of *exalgine.*

PHENACETIN *p* $C_2H_5O{-}C_6H_4{-}NH{-}CO \cdot CH_3$. The conversion of aniline into *p*-amino-phenol by animal metabolism suggested the use of phenacetin, a derivative of *p*-amino-phenol as a drug. Many other derivatives of the above, obtained by substituting hydrogen of the anilido group or by interchanging the acetyl group with other acyl groups have been tried but either the increase in cost or lessening of efficiency has gone against their wide use.

The preparation of phenacetin is possible either by acetylation of *para-phenetedine*, $C_2H_5O \cdot C_6H_4 \cdot NH_2$ or by ethylating *p*-acetamino-phenol, $HO \cdot C_6H_4NHCOCH_3$. The first method may be considered in detail here. *p*-nitro-phenol is dissolved in caustic soda and is ethylated by mixing it with ethyl chloride and heating the mixture under pressure at 90° to 100° for some hours.



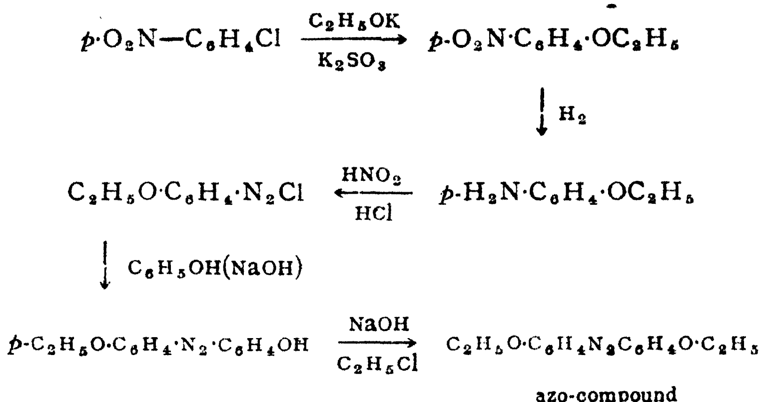
$$HO \cdot C_6H_4 \cdot NO_2 + C_2H_5Cl \longrightarrow C_2H_5O \cdot C_6H_4 \cdot NO_2 + HCl$$

The *p*-nitro-phenetole is then reduced by heating it with *Fe* and hydrochloric acid to give *p*-phenetedine; the latter is then acetylated with $(CH_3CO)_2O$ to give phenacetin.



$$p\text{-}C_2H_5O{-}C_6H_4NO_2 \rightarrow p\text{-}C_2H_5O \cdot C_6H_4NH_2 \rightarrow \underset{\text{phenacetin}}{C_2H_5O{-}C_6H_4NH{-}COCH_3}$$

p-phenetedine may also be prepared by the following methods:

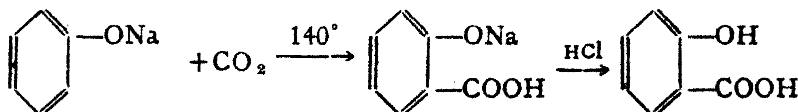


The azo-compound is then reduced to give two molecules of *p*-phenetedine which is acetylated to phenacetin.

It exists in white glistering crystalline form (m. p. 134°C). It is soluble in hot water. It is indicated both in fevers as well as in neuralgia and rheumatism; it acts both as a febrifuge and as analgesic.

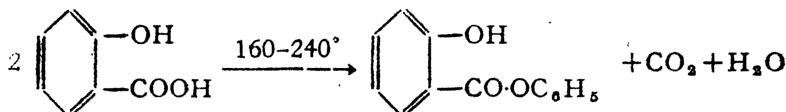
Recently, phenocoll, $p\text{-}C_2H_5O{\cdot}C_6H_4{\cdot}NH{\cdot}CO{\cdot}CH_2NH_2$, obtained by the action of NH_3 on bromo-acetyl phenetedine has been found to possess greater analgesic action.

SALICYLIC ACID AND ITS DERIVATIVES:—Salicylic acid and the salicylates are known to lower the temperature in rheumatic and other fevers and are also very valuable for the relief of rheumatic and other kinds of physical pain. Salicylic acid is manufactured on a large scale by Kolbe-Schmitt synthesis. Dry sodium phenate is heated with dry carbon dioxide at 140° in an autoclave, under pressure giving a sodium derivative of phenol *o*–carboxylic acid from which free acid is precipitated by hydrochloric acid :—

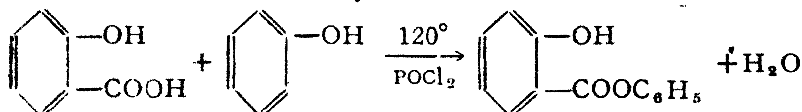


One defect in the medicinal use of salicylic acid as well as its sodium derivative is that it produces unpleasant gastric disturbances. However, in certain derivatives of the acid the above defect is overcome, of which the most important are salol and aspirin.

Salol or phenyl-salicylate is prepared by heating the acid itself in absence of air and with the removal of water formed in the course of the reaction, by distillation :—



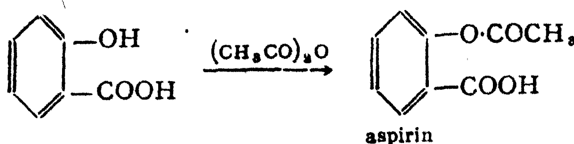
It is also prepared by heating Na-phenate and Na-salicylate in presence of an acid chloride as phosphorus oxychloride or $COCl_2$, which facilitates the process of esterification :—



Nenki introduced salol as an effective intestinal antiseptic; for it eliminated the objectionable secondary effect of salicylic acid. Salol which is an ester passes through the stomach unhydrolysed where conditions are acidic, due to the hydrochloric acid and hence, salicylic acid is not released to bring about gastric disturbances; however, on reaching the duodenum, where alkalinity of the pancreatic juice hydrolyses the salt gradually into sodium salicylate and phenol, these active compounds are available, where they are needful for a biological functioning, without their objectionable toxic effect. Administration of compounds on the above basis, is known as "Salol principle" or "Nenki principle."

Salol is a crystalline compound : m.p. 42 to 43°. Salol is both an antipyretic and an antiseptic. It is indicated in intestinal fermentation and rheumatism. It is also used as an external antiseptic.

Aspirin or acetyl-salicylic is obtained by heating salicylic acid with excess of acetic anhydride in benzene solution at the lowest possible temperature conditions.

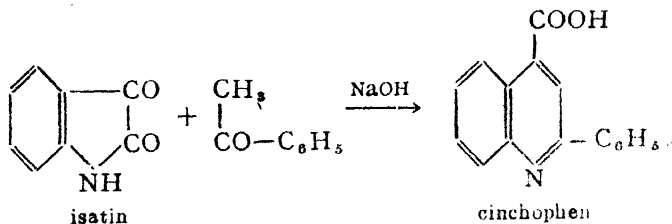


aspirin

At higher temperature (90°), the aspirin formed is converted into salicylo-salicylic acid with the liberation of acetic anhydride. In a recent manufacturing process, the acetylation of salicylic acid is effected with diketen $(CH_2=CO)_2$; the yields are good and the product is highly pure.

Aspirin is a crystalline compound m.p. 135 to 136°. It possesses the specific action of salicylic àcid against rheumatism, and in addition, is a household remedy for headaches and colds.

QUINOLINE DERIVATIVES :—Cinchophen or atophan is administered in the case of rheumatic fever, and also as an antipyretic and analgesic. But its use is known to cause liver poisoning; hence its disuse has been recommended. On a large scale, it is obtained by condensing isatin with acetophenone under strongly alkaline conditions.



isatin cinchophen

It is a yellowish powder m. p. 214 to 217°; it possesses a bitter taste.

Local Anæsthetics

Local anæsthetics are compounds which when applied to some parts of the body, produce a localised insensibility to pain, but do not cause loss of consciousness. The first local anæsthetic to be used was cocaine. But as the latter is highly toxic and is also habit-forming, attempts have been made to develop more effective but less toxic substitutes for cocaine. Some of the more important

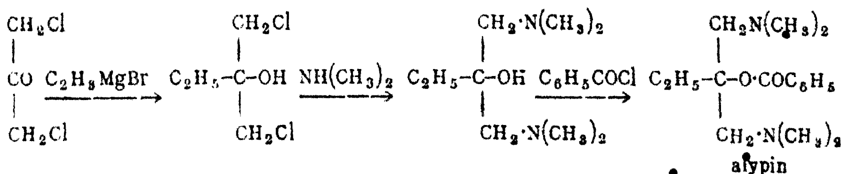
ones in modern use, may be classified into the following fundamental chemical groups:

- (*i*) Piperidine derivatives
- (*ii*) Stovaine and alypin types
- (*iii*) *p*-Amino-benzoic acid derivatives
- (*vi*) Quinoline ethers
- (*v*) Phenetedine derivatives.

PIPERIDINE DERIVATIVES :—The α and β eucaines represent the earliest attempts made to synthesise cocaine substitutes by incorporating the piperidine and the benzoyl groups present in the cocaine molecule, in new compounds. The preparation and properties of these compounds, have been described earlier.

STOVAINE AND ALYPIN :—It was soon found that piperidine nucleus, is not essential for the anæsthetic activity, but on the other hand, it confers toxic properties on the molecules which contain it. These considerations led Fourneau to develop simpler compounds containing the $CH_3—N—C—C—COOC_6H_5$ grouping, which is characteristic of the cocaine and tropacocaine molecules. The most outstanding of such compounds is stovaine. Its preparation and properties have been described earlier.

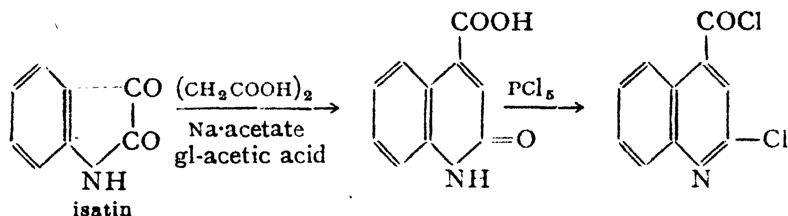
Alypine is another local anæsthetic which resembles stovaine structurally. It is obtained according to the following scheme



It is used in ophthalmic work and in lumber anæsthesia.

PARA-AMINO BENZOIC ACID DERIVATIVES :—It was Ritsert who first, introduced into therapy, ethyl-para amino-benzoate as the *p*-phenyl sulphonate under the name of subcutin. This was followed by several modification of the molecule in the hands of Einhorn and others. The most useful and widely used compounds belonging to this group, are anæsthesine (ethyl *p*-amino benzoate) and novocaine. The preparation and properties of both are described earlier.

QUINOLINE DERIVATIVES :—Quinine possesses local anæsthetic properties and in the form of its hydrochloride, is especially used after rectal operations. A better anæsthetic containing the quinoline nucleus is percain or Nupercain. It is ten times as active as cocaine; it is used in conjunction with adrenaline. It is obtained according to the following series of reactions :



The 2-chloro-cinchoninyl chloride thus obtained is then converted into percain :

COCl

—Cl

N

Br CH_2–CH_2–NH_2

$CONH—CH_2—CH_2Br$

—Cl

N

$NH(C_2H_5)_2$

H_2N–CH_2–$CH_2N(C_2H_5)_2$

$CONH$–CH_2–CH_2–$N(C_2H_5)_2$

—Cl

N

C_4H_9ONa

$CONH$–CH_2–CH_2–$N(C_2H_5)_2$

—$O·C_4H_9$

N

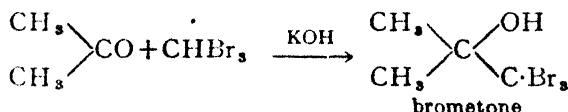
percain

It forms crystals m.p. 97°. It is soluble in water but is very sensitive to alkalis.

Narcotics

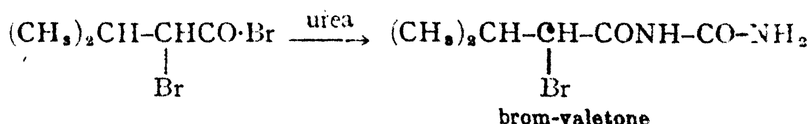
Narcotics are compounds which possess general depressant action on the central nervous system. They can be further classified into: (*a*) Sedatives, (*b*) Hypnotics and (*c*) General anæsthetics. The above classification is only for the sake of convenience, as the action of a drug as a sedative, as a hypnotic or as an anæsthetic depends usually on the dose administered. Pentothal in small doses, may act as a sedative, and in medium doses as a hypnotic, while larger doses of the same, may cause anæsthesia. But in actual practice, it has been found more convenient to restrict oneself to the use of a particular type of compounds as sedatives, to another group of compounds as hypnotics and to others as general anæsthetics.

SEDATIVES :—They cause a milder form of depression. The most widely used sedatives are the bromides and the bromo compounds. KBr is the most commonly used bromide; bromvaletone, brometone, and valero bromine are some of the important organic compounds containing bromine that are used as sedatives. Brometone is obtained by the action of bromoform on acetone in presence of alkali.



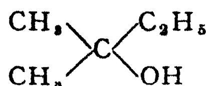
It does not produce bromism.

Bromvaletone is obtained by the action of urea on α-bromo-iso-valerianyl-bromide:



HYPNOTICS :—There are several compounds belonging to different chemical groups, that are used as hypnotics. The most widely in use can be grouped under: (*a*) alcohols and halogenated alcohols, (*b*) aldehydes and ketones, (*c*) barbiturates, (*d*) sulphones and (*e*) urethanes.

ALCOHOLS :—Ethyl alcohol has been used as a sedative and hypnotic for a very long time. But, as its continued use leads to alcoholism, it has been superseded by less toxic and less habit-forming hypnotics. Tertiary amylalcohol:



has a very powerful hypnotic action and is less toxic.

Among the halogenated alcohols, the following compounds, have found extensive applications.

Trichloroethanol, $CCl_3—CH_2OH$,
Tribromoethanol, $CBr_3—CH_2OH$
Chloretone, $(CH_3)_2C$-$OH·CCl_3$

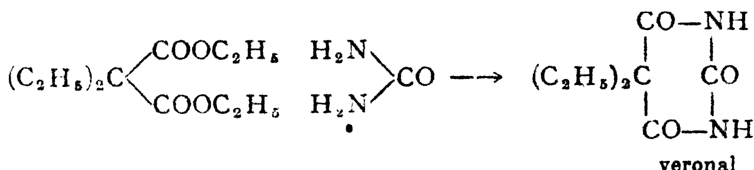
Trichloroethanol is obtained from chloral by reduction with Al-isopropoxide and isopropyl alcohol or the reduction is effected with yeast. Tribromo-ethanol or avertin is similarly obtained from bromal. Chloretone and brometone are very powerful hypnotics; their action is similar to that of chloral hydrate but is less irritant. Chloretone is obtained by adding KOH, to a mixture of acetone and chloroform.

ALDEHYDES AND KETONES :—Aliphatic aldehydes exert a very powerful depressant action on the central nervous system. Paraldehyde $(CH_3—CHO)_3$, obtained from acetaldehyde by polymerisation with a small amount of HCl or H_2SO_4, is a very valuable hypnotic. It is a liquid, B, P. 123° to 126°. It is often used in mental conditions. But it has the disadvantage of a bad taste and a pungent odour and also affects the mucous membranes.

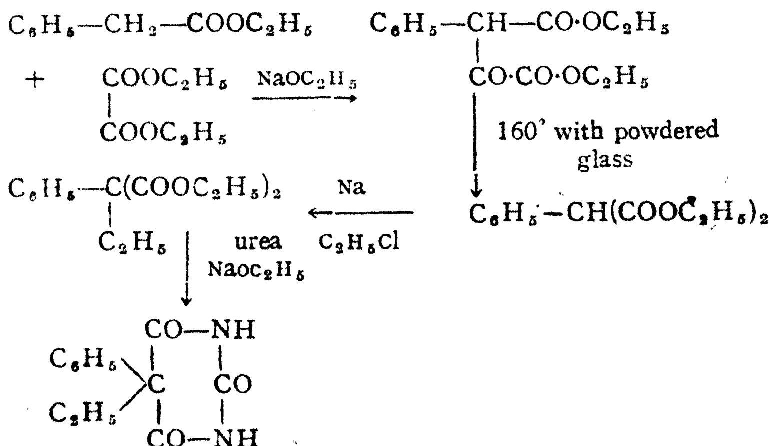
Halogenation of aldehydes, as in the case of alcohols, tends to produce compounds with very valuable hypnotic properties. Chloral hydrate, $CCl_3CH\ (OH)_2$ was, in fact the first synthetic hypnotic, introduced in medicine, as far back as 1869. It is prepared by the action of chlorine on alcohol at ordinary temperature. Chloral alcholate $CCl_3CHOH.OC_2H_5$, is first formed which on distillation with H_2SO_4 gives chloral; the latter on careful hydration gives the crystalline hydrate $CCl_3\ CH(OH)_2$. m. p. 37°.

Ketones also act as hypnotics. The relative narcotic action of this class of compounds depends on the nature of the hydrocarbon radical present, those which contain ethyl group have the most marked effect. True aromatic compounds possess little action, while the mixed ketones are very active. Of these acetophenone, has been much used under the name of hypnone.

BARBITURATES :—Derivatives of cyclic ureides (*q.v.*) have found uses as hypnotics; veronal, the diethyl derivative of barbituric acid was first introduced by Fischer and Merling as a hypnotic in medicine. It is prepared by the condensation of *C*-diethyl malonic ester with urea in presence of $NaOC_2H_5$.

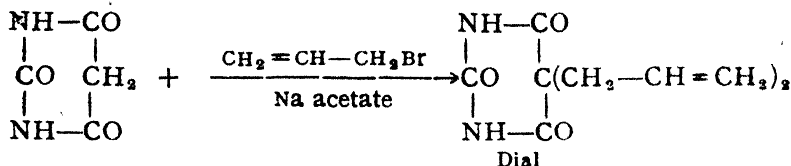


It is a crystalline compound m. p. 191°. Its use has been followed by many other barbituric acid derivatives called by the name of 'Barbitals'; of these, the most commonly used are phenobarbital and dial. Phenobarbital is 5-5′ ethyl-phenyl barbituric acid. It is obtained on a large scale by the following series of reactions :

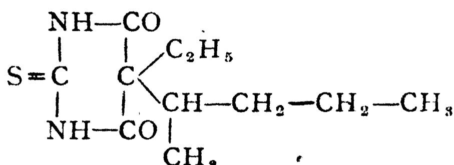


It is usually used in the form of its sodium salt. It is used both as a sedative and hypnotic.

Dial is the diallyl derivative of barbituric acid and is obtained by direct allylation of barbituric acid in aqueous-alcoholic solution, with allylbromide and Na-acetate.

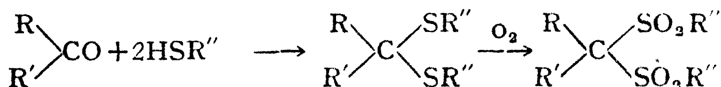


Recently, a thio-barbiturate has been introduced into medicine under the name of pentothal. It has the following structure :



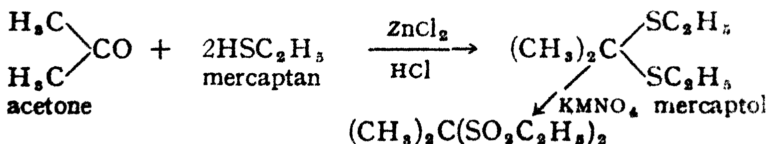
The sodium salt is used. A dilute solution of the Na-salt is injected to produce surgical anæsthesia very rapidly.

SULPHONES :—The group SO_2R is called the sulphone group and the compounds containing these groups are called sulphones. The usual method of preparing these compounds is to condense a ketone with mercaptans, and subsequent oxidation of the mercaptol so formed, changes the group—$S—R$ *to* —SO_2R.



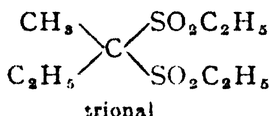
Baumann and Kash thoroughly investigated the physiological action of the derivatives of this group which have a strong hypnotic effect.

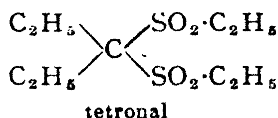
The diethyl-sulphone-dimethyl-methane which is called 'sulphonal' is synthesised by the condensation of acetone with ethyl mercaptan in presence of $ZnCl_2$ and the product is oxidised by excess of permanganate in acidic conditions.



It crystallises in prisms and is sparingly soluble in water m. p. 126.

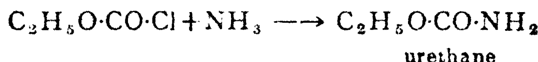
It has been observed that an increase of ethyl groups by substitution for the methyl groups, increases the hypnotic action of the compound. Thus trional and tetronal,


trional

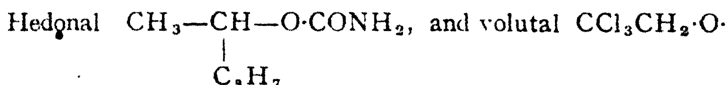

tetronal

possess greater hypnotic activity than sulphonal; while a compound with all methyl groups like $(CH_3)_2C\begin{smallmatrix} SO_2 \cdot CH_3 \\ SO_2 \cdot CH_3 \end{smallmatrix}$ is devoid of any hypnotic activity.

URETHANES :—Urethane or ethyl carbonate is a mild hypnotic with a low toxicity. It is obtained by the action of ammonia on ethyl chloroformate.



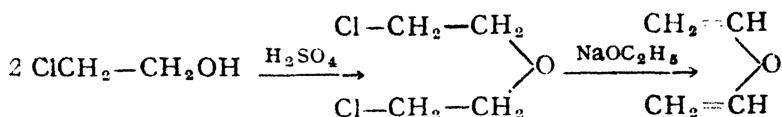
$$C_2H_5O{\cdot}CO{\cdot}Cl + NH_3 \longrightarrow \underset{\text{urethane}}{C_2H_5O{\cdot}CO{\cdot}NH_2}$$



Hedonal $CH_3—\underset{C_3H_7}{\underset{|}{CH}}—O{\cdot}CONH_2$, and volutal $CCl_3CH_2{\cdot}O{\cdot}CONH_2$ are other urethanes used as hypnotics.

GENERAL ANÆSTHETICS :—They are compounds used to produce unconsciousness and muscular relaxation, sufficient to allow the performance of surgical operations. It was Morton in America, who introduced ether anæsthesia for surgical purposes. This was followed by the use of chloroform by Simpson, as a general anæsthetic to relieve labour pains. Ether and chloroform are used as inhalation anæsthetics even today on a large scale. However they possess certain disadvantages: ether is inflammable and also forms peroxides which are dangerously explosive; chloroform, on the other hand, is slowly decomposed in presence of air and light to phosgene ($COCl_2$), which is a highly toxic compound. Other compounds which have been introduced into surgical practice as inhalation anæsthetics are; ethylene, divinyl ether and cyclopropane. In the case of ethylene, a high concentration (85-90%) of the gas, with

a high percentage of oxygen is necessary to produce surgical anæsthesia. Such an anæsthetic mixture however, is highly explosive. It was Leake, who conceived the remarkable idea that a very useful anæsthetic might be obtained by a combination of the molecule of ethylene and ether. Divinyl ether, $CH_2=CH-O-CH=CH_2$ was the expected anæsthetic. It is synthesised from ethylene chlor-hydrin as follows :



This compound has a few distinct advantages over ether; the induction period is much shorter, and the recovery is quick. The disadvantages are that it shows a tendency to undergo oxidation and polymerisation; hence it requires to be stabilised with antioxidants like α-phenylamino-naphthalene. Its use occasionally causes hepatic injury. Cyclopropane was introduced as an inhalation anæsthetic as late as 1930. It is obtained from 1·3 dichloro propane,—a by-product of the chlorination of propane, by the action of metallic zinc. It is a gas and produces surgical anæsthesia in a concentration of 26%. In combination with avertin it promises to be the near ideal general anæsthetic. It possesses a low toxicity and a high therapeutic index; its only disadvantages are that it forms with oxygen a highly explosive and inflammable mixture. Recently, cyclopropyl ethers: the methyl and the ethyl ether have been introduced as surgical anæsthetics.

Antiseptics

It was in 1867 that Lister introduced the use of phenol as as antiseptic *i. e.* a compound which inhibits the growth of bacteria and thus prevents the sepsis of wounds. But phenol also acts as a general proto-plasmic poison, causing damage to the other living tissues as well.

At present, hundreds of compounds, have been developed, which act as antiseptics in concentrations, which cause no damage to the tissues. An ideal antiseptic must possess great potency against all the bacteria, but must not be toxic and irritating to the living tissues.

Penicillin—an antibiotic is probably the nearest approach to an ideal antiseptic. It however affects only a few types of bacteria, while other types completely resist its action.

The antiseptic power of a compound is measured by two standard tests: the Rideal-Walker test and the Chick-Martin test, and is expressed in terms of the phenol coefficient.

$$\text{Phenol coefficient} = \frac{\text{germicidal dilution of the comp.}}{\text{germicidal dilution of phenol}}$$

(The standard time employed is ten mins.)

The modern synthetic antiseptics can be classified into the following chemical types:

(*a*) Phenol and its derivatives

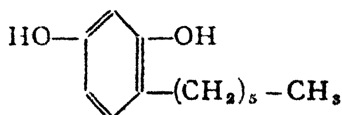
(*b*) Benzoic acid and its derivatives

(*c*) Halogens, chloramines and other halogenated compounds.

(*d*) Synthetic dyestuffs.

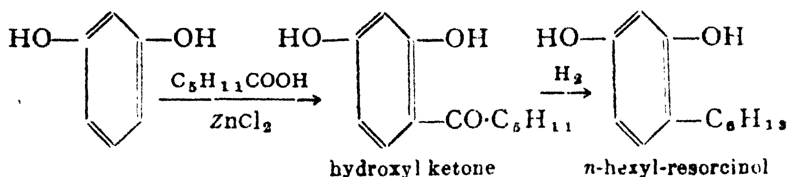
(*a*) PHENOL and ITS DERIVATIONS:—Phenol itself possesses antiseptic properties but it has a strongly irritant action. Physiological action, increases with entrance of *OH* groups in the nucleus, as in catechol and pyrogallol. Hence, to tone down the toxic action, alkyl groups are introduced in the phenol, giving rise to cresols, which are better antiseptics than phenol. However their insolubility in water has led to their use as emulsions either in hard or soft soap. Such a solution of cresols in soft soap is known as Lysol.

The poly hydroxy phenols have not much therapeutic value because of their irritant action. However, some alkyl derivatives have been synthesised from them of which "hexyl resorcinol" is an important member. It has the following structure:—



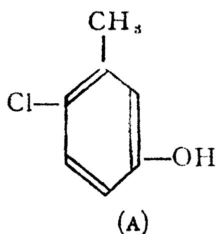
It is obtained by heating resorcinol and caproic acid (C_5H_{11}–$COOH$) in the presence of $ZnCl_2$. The resorcyl–amyl–ketone is first

formed. The ketone is then reduced by Clemmensen's method by amalgamated zinc and hydrochloric acid to the alkylated hydroxy -benzene derivative.

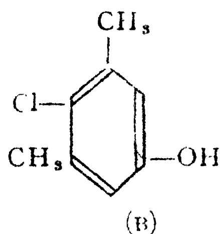


It is a white solid but is sold in solution with glycerol and water, which has a surface tension 37 and hence called S.T. 37. The compound possesses strong antiseptic properties (45 times as active as phenol) and is a urinary antiseptic and effective for washing dental and surgical instruments.

Recently, chlorinated phenolic compounds are finding applications as powerful and valuable antiseptics. Chlorocresol (A) and chloroxylenol (B)



(A)

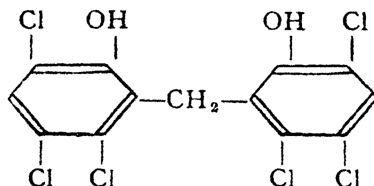


(B)

are powerful, antiseptics, with very little toxicity. The well-known antiseptic *Dettol* is a dilute alcoholic solution of chloroxylenol, together, with terpineol, and a saponaceous solvent. Etherification of the phenolic hydroxyl groups, greatly decreases the antiseptic action of the parent phenol, and increases the toxicity. Hence very few phenolic ethers, except guaiacol have found any use as antiseptics.

The nitro and sulphonic acid derivatives of phenol show more powerful antiseptic activity than phenol; especially, the ortho derivatives are very effective. Picric acid—a trinitro phenolic derivative and *ortho* and *para* phenol sulphonic acids are used as antiseptics.

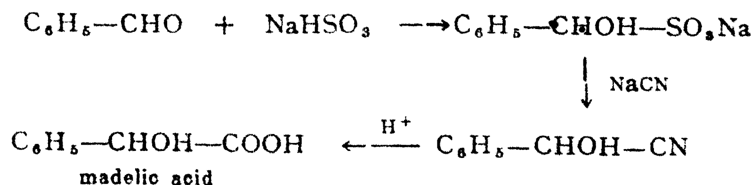
The hydroxy derivatives of polycyclic systems and their chlorinated compounds are introduced as very valuable antiseptics. Hexachlorophen is one of the most successful of such compounds. It has the structure :



Thymol, prepared synthetically from *p*-cymene is a more efficient antiseptic than phenol ; chlorothymol is also an antiseptic ; thymol is a remedy for hookworm disease. β-naphthol is also finding some use as an internal antiseptic.

(*b*) BENZOIC ACID AND ITS DERIVATIVES :—The weak organic acid, benzoic acid has a distinct bactericidal action. It is used in the form of its sodium salt, as a food preservative. Both *p*-hydroxy and ortho-hydroxy-benzoic acids have powerful antiseptic properties ; and the latter is superior to the former. However, its usefulness is limited by the fact that salicylic acid and its salts cause gastric irritation. This difficulty has been overcome by the introduction of the esters, instead of the free acid, as the antiseptics. The most widely used esters are the aryl esters, *salol* or phenyl salicylate and *betol*, β-naphthyl-salicylate.

Mandelic acid $C_6H_5—CHOH—COOH$ is a very valuable urinary antiseptic. It acts on most of the Gram-negative bacteria. It is obtained as follows :—



$$C_6H_5—CHO + NaHSO_3 \longrightarrow C_6H_5—CHOH—SO_3Na$$

$$\downarrow NaCN$$

$$\underset{\text{madelic acid}}{C_6H_5—CHOH—COOH} \xleftarrow{H^+} C_6H_5—CHOH—CN$$

(*c*) HALOGENS AND HALOGEN COMPOUNDS :—Halogens Cl_2, Br_2 and I_2 have a strong germicidal action and Cl_2 in high dilution is used even to disinfect city's water-supply. However, as medicinals,

they are strongly irritant and suffer from an unpleasant penetrating smell as is so well marked in iodoform. But many compounds have been synthesised which are free from above defects.

Sodium hypochlorite was formerly used as an antiseptic for wounds in war. It, however, has strong irritating action and hence has been superseded by Dakins solution and eusol in the treatment of war wounds. Dakins solution is obtained by treating bleaching powder with Na_2CO_3 solution and subsequently adding boric acid to the filtrate.

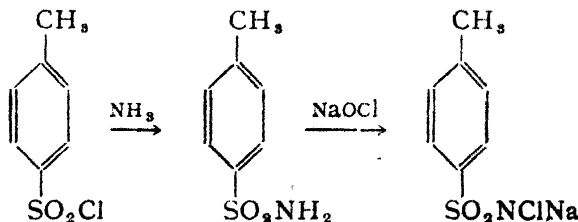
Eusol is prepared by treating bleaching powder with boric acid. The solution contains free hypochlorous acid.



$$2CaOCl_2 + 2H_3BO_3 \longrightarrow 2HClO + CaB_2O_4 + CaCl_2 + 2H_2O$$

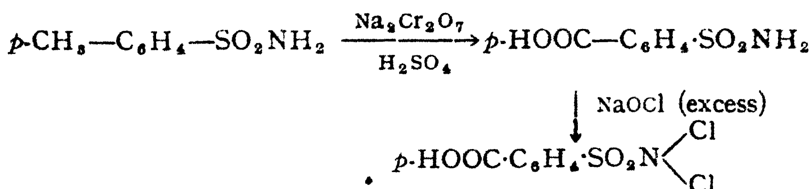
Thus hypochlorous acid appears to be the most effective antiseptic agent and hence other sources of the acid were searched for; the result was the development of organic chlorine compounds, the most important of which are the organic chloramines. Chloramines are compounds wherein the hydrogen atoms of the imino ($NH.$) or amino (NH_2) groups are substituted by chlorine atoms giving chloramine groups as NCl or NCl_2.

CHLORAMINE-T. (*p-Toluene Sulphone chloramide*) :—This compound is prepared from *p*-toluene sulphonyl chloride, which is obtained either as a by-product in production of saccharin, or may be synthesised from toluene which is converted into toluene *p* sulphonic acid, the sodium salt of which on treatment with phosphorus pentachloride gives the required compound. This compound is treated with ammonia to convert it into *p*-toluene-sulphonamide which on gentle warming with sodium hypochlorite gives chloramine-*T* as a mass of colourless crystals :—



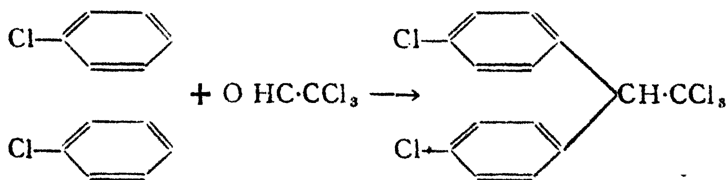
The dichloramine having the structure $CH_3C_6H_4SO_2NCl_2$ is prepared by the action of hypochloric acid on chloramine-*T*. It is a crystalline compound m. p. 83°:- These compounds react with water slowly, to give Cl_2 and hence are powerful disinfectants and were widely used in the last Great War for the irrigation of wounds and are superior to hypoclorite solution by their stability and certainty of composition. They are also non-irritant.

Halazone is closely related to di-chloramine *T*. It is obtained as follows :—



The Na salt is soluble and used for sterlisation of drinking water.

Two of the modern chlorinated compounds used widely as insecticides are D. D. T. and gammexane, or 666 or B.H.C. D.D.T. is obtained by condensing chlorobenzene with chloral in presence of con. H_2SO_4 or chlorosulphonic acid at low temparature (15·20°).



It used in very dilute solutions, and has helped successfully to eradicate the body louse and the malarial mosquito.

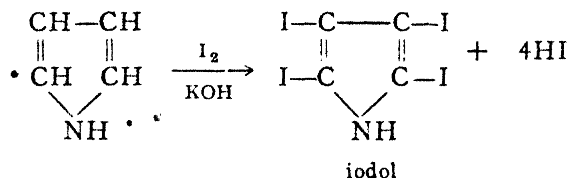
Gammexane is the γ-isomer of 1, 2, 3, 4, 5, 6 hexa-chloro-cyclo-hexane ($C_6H_6Cl_6$). It is obtained by the action of chlorine on benzene in presence of bright or irradiated light, and a small quantity of alkali and at low temperatures :



A mixture of four stereo-isomerides is obtained, which contains 10% to 15% of the active γ-isomer. The mixture is used commercially. γ-isomer is selectively extracted from the mixture with methanol. The pure γ-isomer is called "lindane", it is a solid m.p. 112·5°

Iodine is a powerful antiseptic and is widely used in the form of a tincture "tincture of iodine." However, it is unreliable as a wound antiseptic, if much blood is present. Hence compounds containing iodine have been introduced as antiseptics. The antiseptic properties of iodine compounds are dependent upon the capacity of the compounds to liberate free iodine in contact with the wound, for which purpose, iodine in the compound must be labile and not firmly linked. Iodoform, the most common iodine-bearing antiseptic is prepared by the action of iodine on a mixture of acetone or alcohol in an alkaline solution of sodium hydroxide or carbonate. It is also prepared by electrolysing a solution of potassium iodide with alcohol in a current of CO_2. Though, iodoform possess the properties of a good antiseptic it possesses an objectionable smell and in some persons, causes a definite toxicity syndrome.

Efforts have therefore been made to obtain compounds containing iodine, which can effectively substitute iodoform. Two such compounds are iodol, tetra-iodo-pyrrol and aristol which is dithymol-di-iodide. Iodol is obtained by the alkaline iodination of pyrrole in alcohol.



Iodol is an odourless, non-irritating antiseptic.

SYNTHETIC DYES:—Many synthetic dyestuffs possess the property of staining particular tissues and of differentiating between micro organisms. This selectivity of action has been utilised by the medicinal chemist, to use them as therapeutical agents,

especially as parasiticides. Some of them. which are widely used belong to different chemical types. Thus we have:

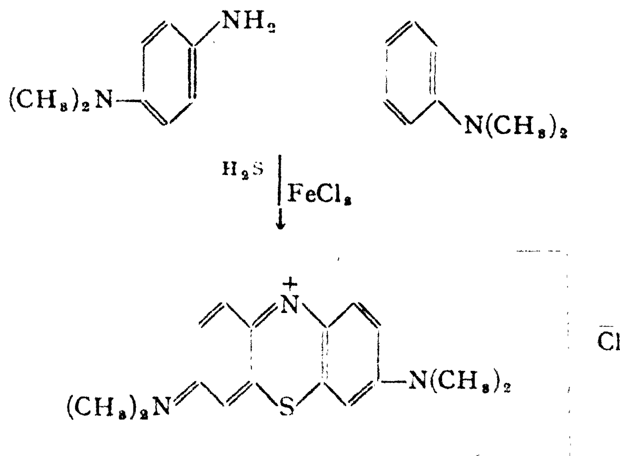
(a) Triphenylmethane dyes — Methyl violet, crystal violet, malachite green.

(b) Acridine dyes ... ... Acriflavine and proflavine.

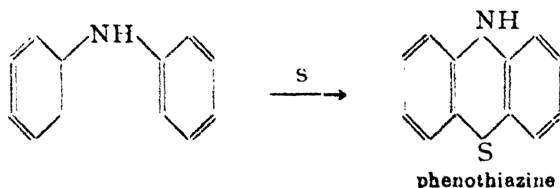
(c) Thiazine dyes ... ... Methylene blue, pheno-thiazine.

(d) Azo dyes.

Methylene blue was the first synthetic dye to be used as an antiseptic. It is obtained by the action of H_2S on a mixture of dimethyl-*p*-phenylene-diamine and dimethylaniline, in presence of a mild oxidising agent like $FeCl_3$ in HCl.



It is a mild antiseptic, and specially finds use as a genitourinary antiseptic. A much valuable urinary antiseptic is phenothiazine. It is obtained by heating under catalytic conditions a mixture of S and diphenyl-amine.



Methyl violet is obtained on a large scale by heating together a mixture of dimethylaniline, $CuSO_4$, NaCl, phenol and water, for 6 to 8 hours. It is probably a mixture of polymethylated para-rosaniline.

CRYSTAL VIOLET is the hexamethyl derivative of para rosaniline. It is obtained by heating dimethyl aniline (a large excess) with $COCl_2$ in presence of $ZnCl_2$. Michler's ketone is first formed, which further condenses with dimethyl aniline to give the dyestuff.

$COCl_2$ + 2 $C_6H_5N(CH_3)_2$ ⟶ $CO[C_6H_4N(CH_3)_2]_2$

michler's ketone

↓ $POCl_3$

$HOC[C_6H_4N(CH_3)_2]_3$

base of crystal violet

$\xrightarrow{HCl}$ $[(CH_3)_2N{-}C_6H_4]_2C{=}C_6H_4{=}\overset{+}{N}(CH_3)_2\ Cl^-$

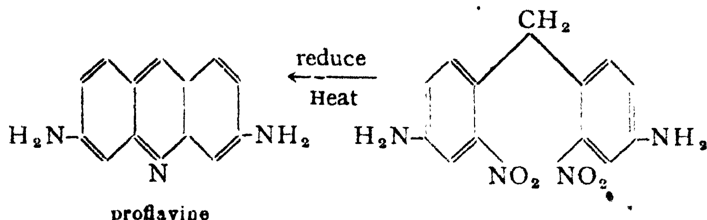
crystal violet

It is a powerful antiseptic with a selective action on Gram-positive bacteria.

PROFLAVINE :—It is 2, 8 diamino acridine. It is obtained by the following method. Aniline is condensed with formaldehyde to give a diphenylmethane derivative, which on heating further with aniline hydrochloride is converted into pp'-diamino-diphenyl-methane. The latter is then nitrated with nitric acid and H_2SO_4 to give a dinitro-derivative. On reduction with tin and HCl, a tetramino derivative is formed, which on heating with $SnCl_4$ in an autoclave gives the dyestuff. Schematically :

$CH_2O + 2\,C_6H_5NH_2 \longrightarrow H_2N{-}C_6H_4{-}CH_2{-}C_6H_4{-}NH_2$

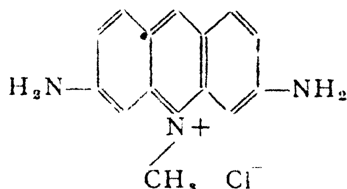
↓ HNO_3



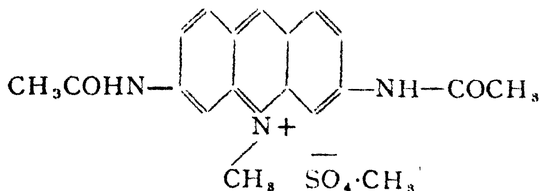
proflavine

In a recent method, proflavine is obtained by heating a mixture of *m*-phenylene diamine, glycerol, $ZnCl_2$ and oxalic acid at 190° for 2 hours. The dyestuff is usually used in the form of its sulphate.

ACRIFLAVINE the metho-chloride of proflavine has been assigned the following structure:

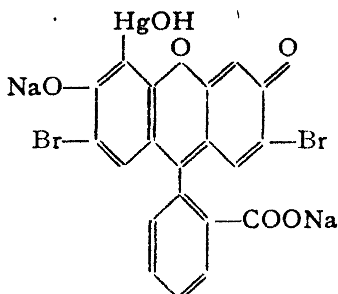


It is obtained from proflavine by first acetylating it and subsequently methylating it with methyl sulphate in nitrobenzene solution. The compound thus formed has the structure:



on boiling with HCl, deacetylation takes place and on cooling the metho-chloride separates out.

Lastly a few metallo-organic compounds have found wide use as antiseptics. Mercuro-chrome is one of such organic mercurials introduced into modern therapeutics. It is obtained by the mercuration of dibromo-fluorescein with mercuric acetate, in alkaline conditions. It has been assigned the following constitution:



It is said to be an effective antiseptic in presence of proteins and is non-irritant. It is also a good drying agent for burns.

Colloidal suspensions of silver, silver chloride and silver iodide have been employed as antiseptics on mucous membranes. A few of the zinc compounds *e.g.* zinc sulphate, zinc oxide and zinc soaps have been used as very valuable moderate antiseptics.

Chemotherapy

Though the word means the treatment of disease by chemicals in its restricted connotation, it implies the treatment of parasitic diseases by synthetic chemicals, for the purpose of destroying the specific parasites of these diseases. The foundation of chemotherapy was laid by Ehrlich. In the beginning, diseases caused by spirochetes and protozoa were cured by such syntheticals, and no progress was made against bacilli or cocci till the discovery of the sulpha drugs.

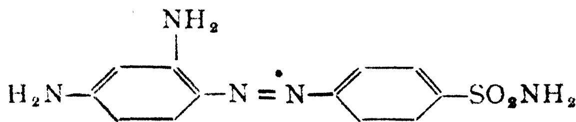
It has now been established that human diseases are caused by the presence of parasites which include (*a*) protozoa, (*b*) bacteria and fungi and (*c*) the filterable viruses. In the treatment of any disease, a chemotherapeutical agent is sought which will kill the parasites, without endangering the life of the host. Several such compounds have been developed from time to time to fight successfully a large number of the common human diseases. These compounds can be classified as :

(*a*) anti-malarials
(*b*) anti-bacterials
(*c*) anti-biotics
(*d*) trypanocides, and
(*e*) anti-histamines

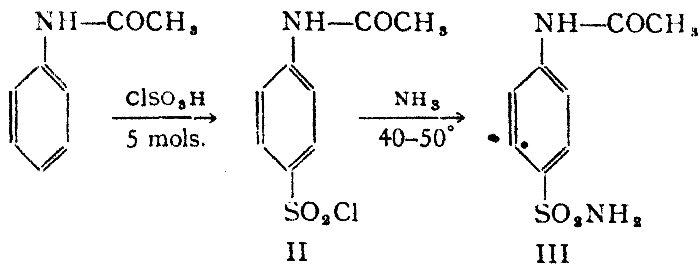
ANTIMALARIALS :—Malaria is one of the most widely spread protozoal diseases. For centuries quinine has been the remedy for this disease, but during the last two decades, attempts have been made to develop effective synthetic substitutes. The effective of such synthetic drugs are plasmochin, atebrin, chloroquin and paludrine.

Sulpha Drugs

ANTIBACTERIALS :—Many of the human diseases are of bacterial origin and no progress was made in the treatment of these diseases *e.g.* pneumonia, meningities, septicæmia etc. till the epoch-making discovery of the sulpha drugs. In 1935, Domagk announced that the azo dye, *prontosil* :



obtained by coupling diazotised sulphanilamide with *m*-phenylene diamine, was remarkably effective in curing experimental streptococcal and staphylococcal infections in test animals. Subsequently Trefouels* found that prontosil breaks down in the body to form sulphanilamide (p-$H_2N—C_6H_4—SO_2NH_2$) and the latter is as effective as prontosil in the cure of the infections. It is obtained on a large scale by the chlorosulphonation of acetanilide and subsequent reactions according to the following scheme :

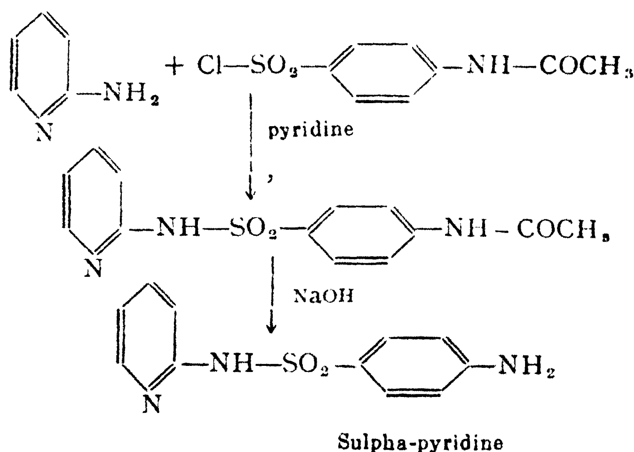


The last compound on hydrolysis with 15% to 20% HCl at 100° gives the hydrochloride, which on treatment with NaOH gives sulphanilamide or alternatively, acetyl-sulphanilamide (III) may be hydrolysed with alkali and subsequently acidified. (The compound

II is the N^4 acetyl sulphanilyl-chloride (ASC) is a very useful intermediate employed in the preparation of the sulpha drugs).

Sulphanilamide is a crystalline compound m. p. 164° to 165°. It is relatively more toxic and has certain disagreeable aftereffects. Hence it has been superseded by less toxic and more effective derivatives. In the years that followed this epoch-making discovery, several hundreds of sulphanilamide derivatives have been synthesised and tested for their activity against the common streptococcal and staphylococcal infections. But only a few (eight or nine) have been found to possess great activity and are used in large amounts in modern medical practice. They are : sulpha-pyridine, sulpha-thiazole, sulpha-diazine, sulpha-merazine, sulpha-methazine, sulpha-guanidine, succinoyl-sulpha-thiazole, phthaloyl sulpha-thiazole, and marfanil. They are referred to as the wonder drugs.

SULPHA-PYRIDINE (M.B. 693). It is obtained by condensing ASC with 2-amino-pyridine in pyridine as the solvent.

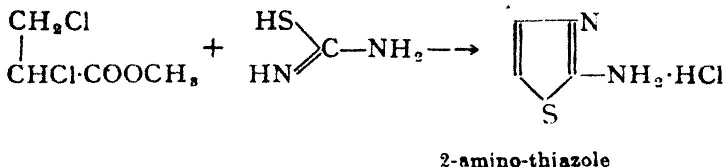


Sulpha-pyridine

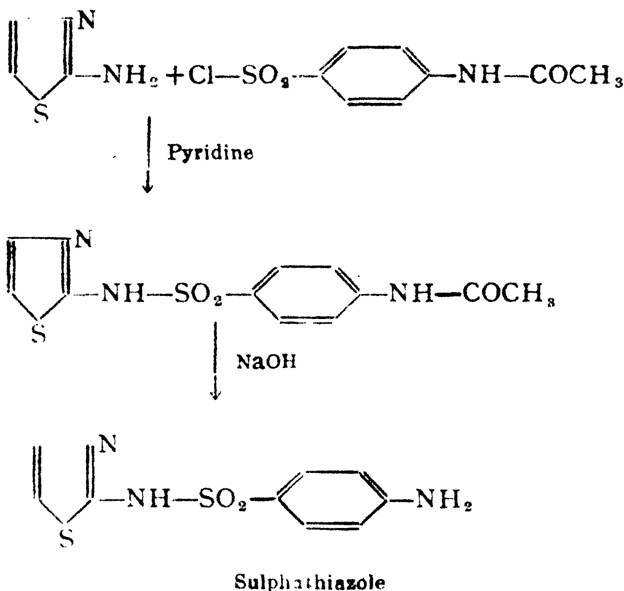
(2-aminopyridine is obtained by the action of $NaNH_2$ on pyridine, in toluene or dimethyl aniline at 120°C.). However it is toxic and is practically abandoned in modern medical practice.

SULPHA-THIAZOLE (cibazol). It is prepared by condensing ASC with 2-amino-thiazole. The latter is obtained by condensing

thiourea with 1, 2-dichloroethyl ether or with 1, 2-dichloroethyl acetate (from vinyl acetate and Cl_2 at low temp.)

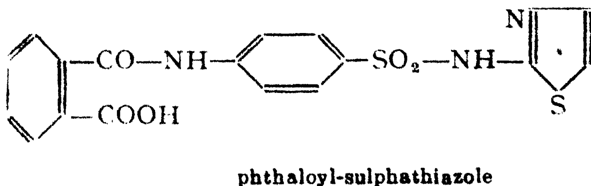


2-amino-thiazole

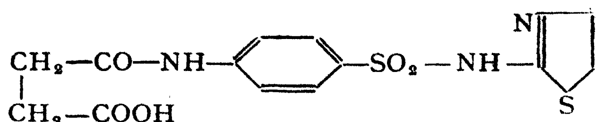


Sulphathiazole

The N[4] phthaloyl an N[4] succinoyl derivatives of sulphathiazole have been found to possess specific activity. They are obtained by heating sulpha-thiazole with phthalic and succinic anhydrides respectively, in alcoholic solutions.

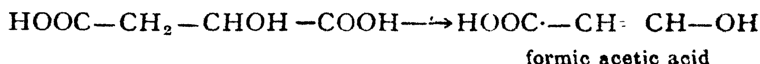


phthaloyl-sulphathiazole

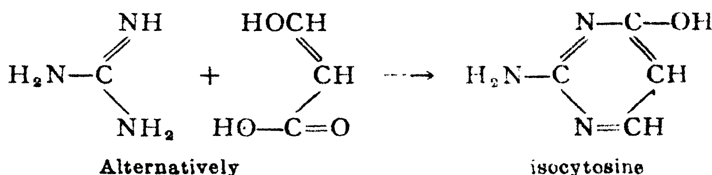


succinoyl-sulphathiazole

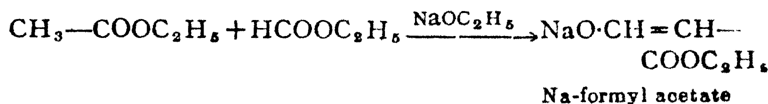
SULPHA-DIAZINE :—It is as potent as sulphathiazole and posses-ses no toxic side reactions. It is obtained on a large scale by condensing ASC with 2-amino-pyrimidine in presence of pyridine and subsequently hydrolysing the product with alkali; 2-amino-pyrimidine is prepared from isocytosine. The latter is obtained from the condensation of guanidine with formyl acetic acid or its ester (Na derivative).



formic acetic acid

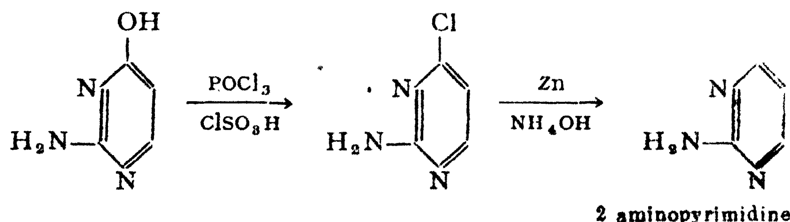


Alternatively isocytosine



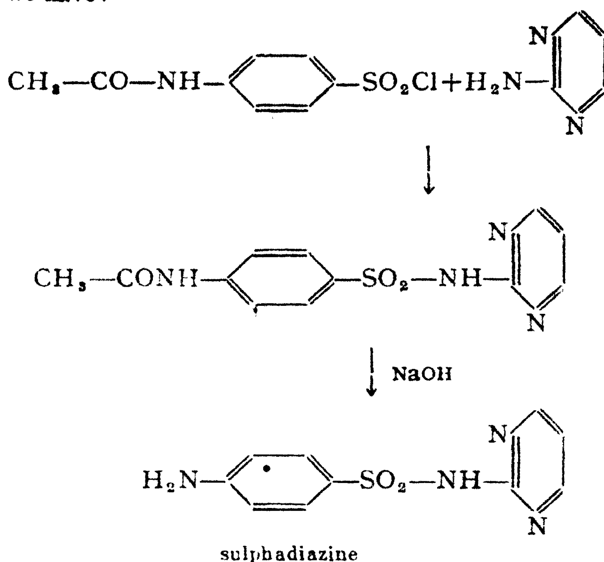
Na-formyl acetate

The latter on condensation with guanidine gives isocytosine. It is then converted into 2-amino pyrimidine as follows :



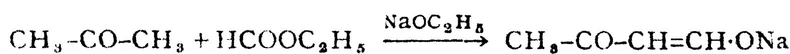
2 aminopyrimidine

Finally we have:

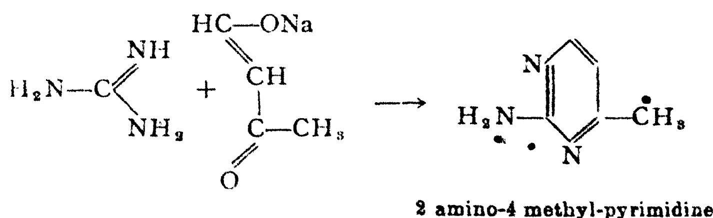


sulphadiazine

SULPHA-MERAZINE:—It is the 4-methyl (pyrimidine) derivative of sulphadiazine and is obtained by an analogous method from ASC and 2-amino-4-methyl-pyrimidine. The latter is prepared by the condensation of formyl acetone with guanidine.

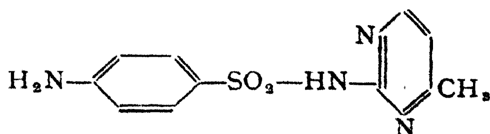


$$CH_3\text{-}CO\text{-}CH_3 + HCOOC_2H_5 \xrightarrow{NaOC_2H_5} CH_3\text{-}CO\text{-}CH{=}CH{\cdot}ONa$$

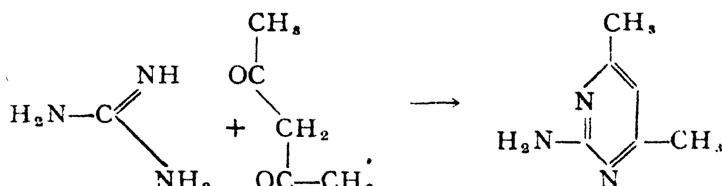


2 amino-4 methyl-pyrimidine

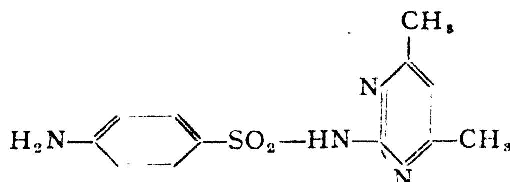
Sulpha merazine has the structure:



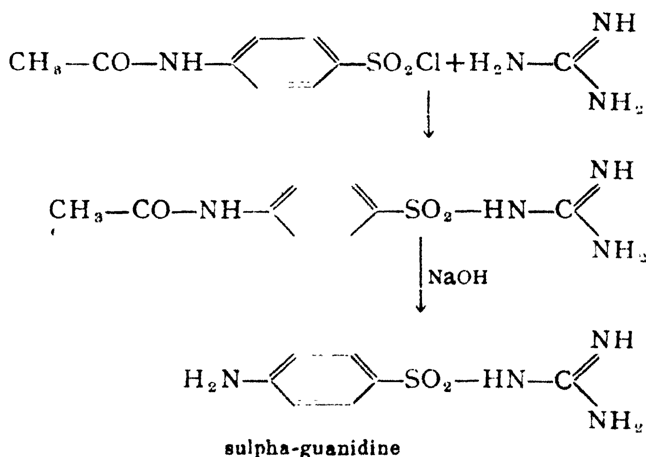
SULPHAMETHAZINE is the corresponding di-methyl derivative and is obtained in an analogous way. 2-amino-4-6-dimethyl pyrimidine is prepared by the condensation of guanidine with Na-derivative of acetyl acetone:



It is converted into the drug in the usual way. The drug has the structure:



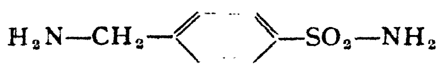
SULPHA-GUANIDINE :—It is obtained by condensing ASC with guanidine and subsequently hydrolysing under alkaline conditions.



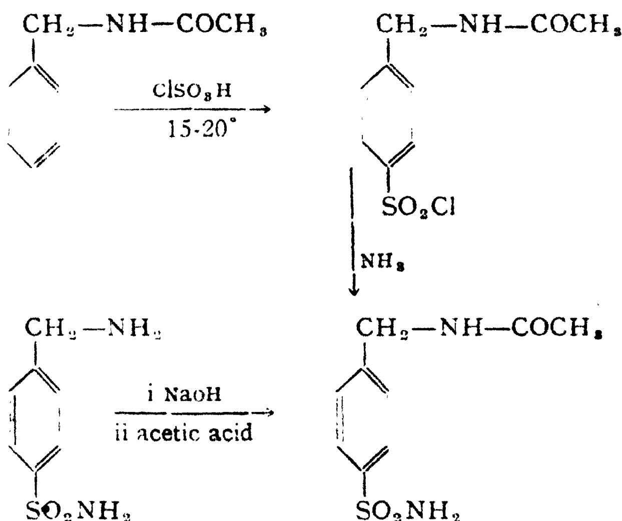
sulpha-guanidine

This drug is especially valuable in the treatment of intestinal infections. It is absorbed very slowly and has no toxic after effects.

MARFANIL is a sulpha drug with structure :

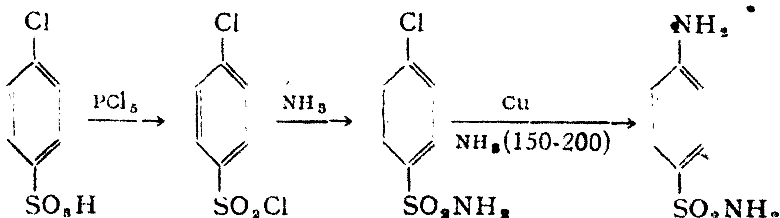


It is prepared according to the following scheme :



This drug has been of value in the treatment and cure of gas gangrene.

In Russia, the sulpha drugs are obtained by a different method. It involves the amination of *p*-chlorobenzene sulphonamide derivatives. The starting material is *p*-chlorobenzene sulphonic acid.



The amination requires vigorous control of (i) temperature, (ii) concentration of NH_3 and (iii) the amount of catalyst. In an analogous way, the other sulpha drugs have been obtained.

All the active sulpha drugs carry a free NH_2 group in position 4; if the N^4 is substituted by a group which is not eliminated by a metabolic change in the body, the activity of the compound is entirely lost. With phthaloyl and succinoyl derivative it is believed that these groups are rapidly eliminated. Further, it is found that all the active drugs contain the grouping :



According to Woods and Fildes theory, the sulpha drugs interfere with the absorption of *p*-amino-benzoic acid—an essential metabolite of the bacteria. The sulpha drug which is structurally analogous to the *p*-amino benzoic acid, is taken up by the bacteria: it cannot perform the essential function of the *p*-amino-acid and hence the bacteria are killed of inanition.

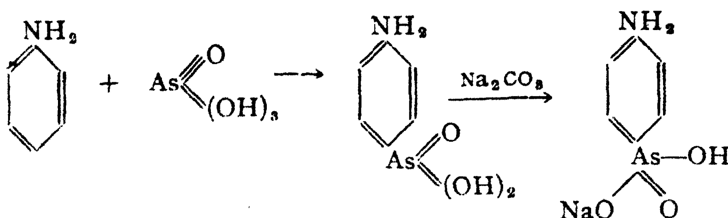
Trypanocides

Trypanosomiasis is one of the dreadful African diseases. It is caused by trypanosomes and is transmitted by tsetse flies. Syphilis is also a protozoal disease caused by spirochætes. It was Ehrlich and Shiga who in 1904, made the discovery that the azodye Trypan Red, could cure the infections caused by trypanosomes, spirochætes and malarial parasites, in mice. But it was found to have no value in the treatment of human trypanosomiasis. At about the same time, Thomas and Breinl showed that atoxyl was very active against trypanosomes.

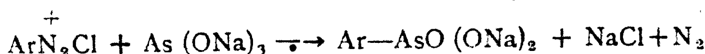
ATOXYL :—It is related to aryl arsonic acids, which have the general formula Ar-$AsO{\cdot}(OH)_2$ and in which As is pentavalent. These well-defined crystalline compounds on reduction yield either arsine oxide ($Ar—As=O$) or arseno-benzene compound ($Ar—As=As—Ar$) or primary arsines $Ar—AsH_2$ in which behaviour they resemble the nitro compounds which give nitroso or diazo or amino derivatives. They possess acid properties and form stable alkali salts.

Atoxyl is the mono sodium salt of arsanilic acidic-*p*-amino phenyl arsonic acid. Arsanilic acid was obtained by heating aniline n excess with arsenic acid at a temperature of 163° to 184° and the

product so formed was extracted with sodium carbonate to give the sodium derivative which is subsequently recrystallised.



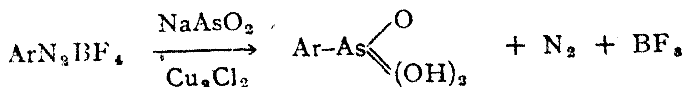
The above reaction is known as Béchamp reaction; it is limited to phenols and aromatic amines. Hence it is now replaced by the more versatile Barts' reaction. The latter consists in the action of sodium arsenite on diazonium halides, in presence of Cu salts.



In the Scheller's modification of the Bart's reaction, the diazonium salt is condensed with $AsCl_3$ in acetic acid solution and the chloro-derivative formed is hydrolysed in presence of $Na_2S_2O_4$.



A still recent modification is that Aryldiazo–fluo–borate is decomposed with Na–arsenite in presence of Cu_2Cl_2 and alkali.



Atoxyl is now obtained in much bettter yields by the application of this reaction to *p*-nitraniline. *p*-nitro-phenyl arsonic acid is first formed which is reduced to arsanillic acid with Na-arsenite.

Atoxyl forms colourless crystals which are soluble in water giving neutral reaction to litmus. It is used against syphilis, relapsing fever, anæmia and skin diseases. It is found that atoxyl is devoid of action on parasites *in vitro* but is very effective in *vivo* which indicates that the compound suffers change through metabolism of the body. Of the many theories formulated to account for the above behaviour, the most important is that, the pentavalent arsenic

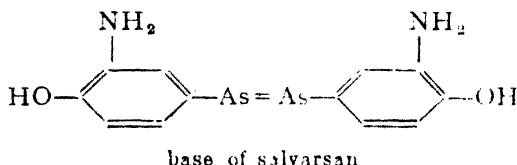
in atoxyl is changed into trivalent arsenic compounds of which are found to be very effective in their parasiticidal action, even *in vitro*.

Ehrlich was thus led to make a systematic search for new compounds of arsenic, in which it was trivalent. This finally led to the development of arsephenamine or salvarsan; as it was number "606" in the series of compounds investigated by him, it is also known as "606." Salvarsan belongs to the class of compounds called arseno-benzenes with the general formula :



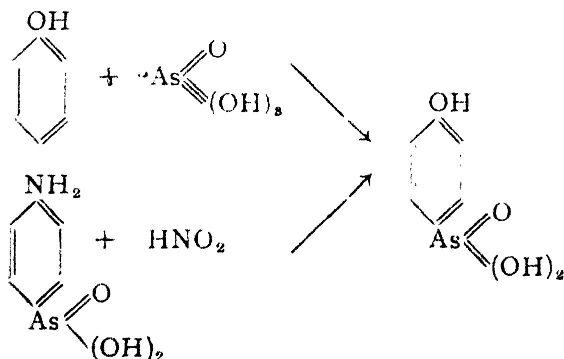
$$Ar—As=As—Ar.$$

These compounds are prepared by the reduction of phenyl arsonic acids. The reduction by metals and concentrated acids yield primary arsines $ArAsH_2$ while, mild reducing agents like sulphurous acid (H_2SO_3) or hydroiodic acid (HI) give arsine oxides $ArAs=O$, and sodium hydro sulphite $(Na_2S_2O_4)$ reduces the acids to arseno compounds. Salvarsan is the dihydrochloride of *pp'*–dihydroxy–*mm'* –diamino arseno–benzene having the following formula :

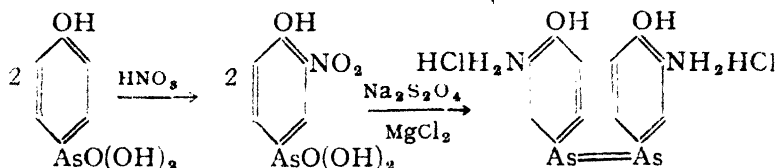


base of salvarsan

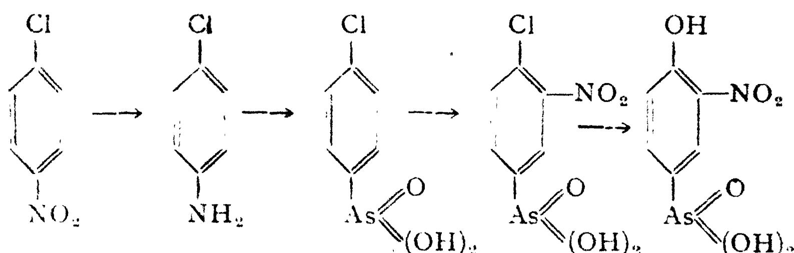
It is obtained from *p*-hydroxy-phenyl-arsonic acid which is prepared either by the action of hydrated arsenic oxide (arsenic acid) on phenol or the action of nitrous acid on arsanilic acid.



The nitro group *ortho* to the hydroxyl and *meta* to the arsonic group, is then introduced in this acid by the action of nitric acid and the nitro compound so formed is reduced by sodium hydrosulphite and magnesium chloride yielding dihydroxy-diamino-arsenobenzene. This base is dissolved in methanol and hydrochloric acid also dissolved in methyl alcohol is added, care being taken to exclude air, when salvarsan separates as a crystalline substance.

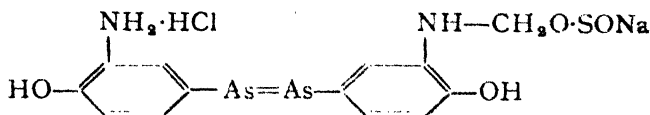


3 nitro-4-hydroxy-phenyl arsonic acid is obtained from *p*-chloro-nitro benzene as follows :



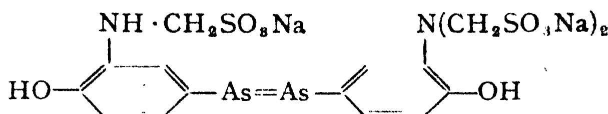
which is then reduced to salvarsan.

This yellow crystalline substance is soluble in water, methanol glycerol, and is readily oxidised by air to *p*-amino phenyl oxide and hence is preserved in sealed tubes in an atmosphere of an inert gas. Its solution has a strongly acid reaction, which is a disadvantage in its administration and the solution has to be neutralised exactly with alkali before intravenous injections. However this difficulty is eliminated by using a sulphoxylate derivative of salvarsan which is a soluble neutral derivative and is called Neo-salvarsan or "914." It has the following formula.



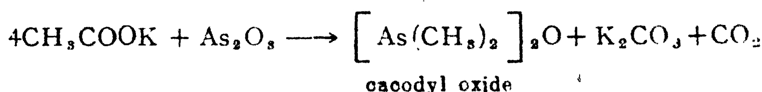
It prepared by the action of sodium formaldehyde-sulphoxylate on an aqueous solution of salvarsan.

A still better drug is sulpharsephenamine; it is obtained by treating the dihydro chloride of salvarsan with CH_2O and $NaHSO_3$.



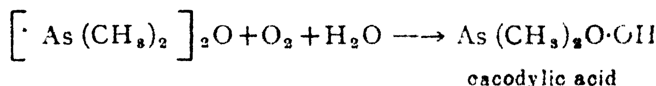
It can be administered intramuscularly.

NA-CACODYLATE is another important modern organic arsenical. Cacodyl oxide is obtained by distilling a mixture containing equal amounts of K-acetate and of As_2O_3 :



$$4CH_3COOK + As_2O_3 \longrightarrow \underset{\text{cacodyl oxide}}{\left[As(CH_3)_2\right]_2O} + K_2CO_3 + CO_2$$

The cacodyl oxide on boiling with water and mercuric oxide, is converted into cacodylic acid :

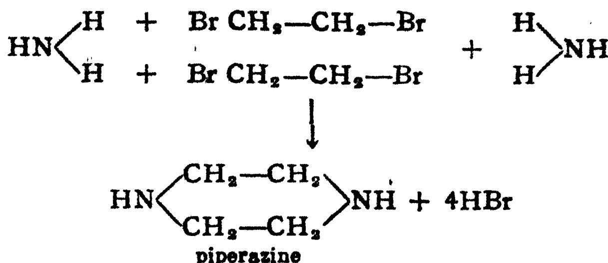


$$\left[As(CH_3)_2\right]_2O + O_2 + H_2O \longrightarrow \underset{\text{cacodylic acid}}{As(CH_3)_2O{\cdot}OH}$$

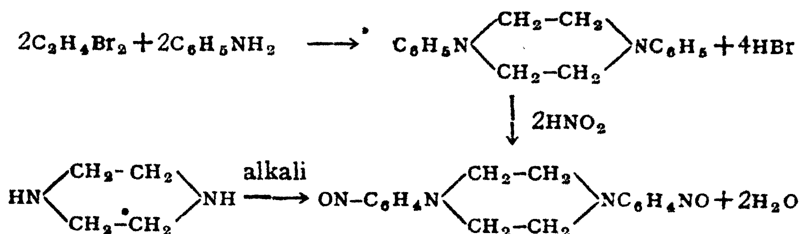
With aqueous NaOH, it gives the Na-salt which is the drug.

DIURETICS AND URIC AND ELIMINANTS :—The most important members of purine group such as caffeine, theobromine, theophyllin and para-xanthine, possess diuretic action and have been discussed in the chapter on Purines. A drug aiming to remedy gout which is due to formation of uric acid in the body must serve two purposes; firstly it must act as a solvent for the uric acid formed and secondly diminish the formation of uric acid in the body. Piperazine is found to dissolve uric acid in *vitro*, though in high concentrations it may fail to do so; on the other hand quinic acid ($C_6H_7(OH)_4{\cdot}COOH$) a tetrahydroxy alicyclic acid which is found in cinchona bark, acts so as to diminish the formation of uric acid. Hence the salt of piperazine with quinic acid—piperazine quinate—will efficiently function for both the purposes mentioned above and this salt is used under the name of Urol or Sidonol.

Hofmann synthesised piperazine by the action of ammonia on ethylene dichloride or dibromide :—



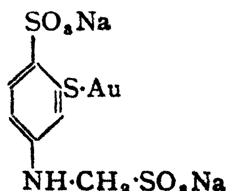
Another method treats ethylene dibromide with aniline, and the product diphenyl-piperazine is converted into dinitroso compound by the action of nitrous acid and the dinitroso derivative on distillation with alkali gives piperazine.



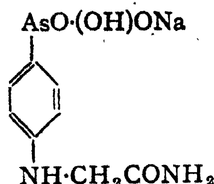
The substance which is crystalline in nature, is extremely soluble in water giving alkaline reaction. It has been found to be a good remedy in cases of gout and rheumatism due to its solvent action on uric acid.

Some other modern important chemo-therapeutic agents are solganal and tryparsamide.

SOLGANAL :—This is a gold preparation used in the treatment of tuberculosis. It has been assigned the following formula :—



Tryparsamide or Tryponarsyl:—It is the *Na* salt of *N*-phenyl glycine-amide-*p*-arsonic acid:—

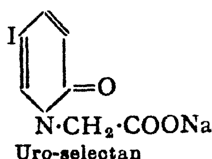


It is extensively used in the treatment of sleeping sickness.

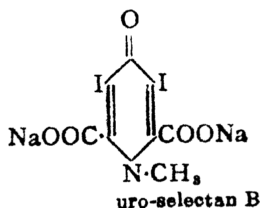
ANTHELMINTICS:—The most common anthelmintics are the natural products *e.g.* thymol, carvacrol etc. However, CCl_4 has been found to give very good results as an anthelmintic.

DIAGNOSTIC REAGENTS:—Recently, a large number of chemical compounds have been used as diagnostic reagents either in Skiagraphy (*i.e.* X-ray examination of the body parts) or in testing the functional activity of bodily organs like the kidney and the liver.

Uroselectan and *Uroselectan B* are the two compounds used for rendering certain tissues *e.g.* nerves and artries opaque to X-rays; they thus find extensive application in X-ray examination. Their structural formulas are:—



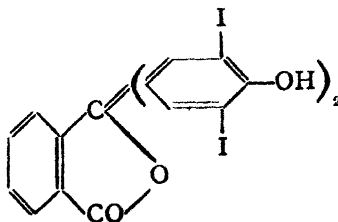
Uro-selectan



uro-selectan B

(The latter compound is related to chelidonic acid)

Tetra-iodo-phenolphthalein obtained by iodinating phenolphthalein finds use in the radiological examination of gall bladder. It has the structure:—

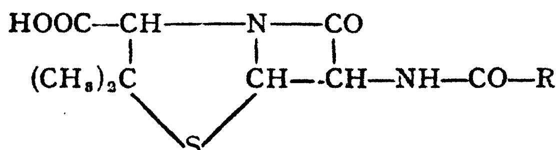


Phenol red and the halogenated phthaleins are employed to test the functional activities of kidneys and the liver. They are rapidly excreted by kidney and are capable of being quantitatively estimated by a relatively simple method *e. g.* colorimetrically.

Antibiotics

Waksman has defined the term as a chemical substance, produced by micro organisms, which is capable of inhibiting the growth and of destroying bacteria and other micro organisms. Further the mode of action of an antibiotic is selective. The first one of the antibiotics to be popularised was *penicillin.* It was discovered by Fleming in 1929, but was isolated from the fermentation liquor, by Florey, Chain and others. The other antibiotics introduced in modern chemotheraphy, are streptomycin, chloromycetin, aureomycin, terramycin, etc.

PENICILLIN :—Actually, there are several penicillins ; the medicinally most effective is penicillin G. The structures of the common penicillins are as follows :



They differ in the nature of the group R.

Penicillins	R =
F or I ...	... $CH_3—CH_2—CH=CH—CH_2—$
F (dihydro)	... $CH_3—CH_2—CH_2—CH_2—CH_2—$
G or II ...	... $C_6H_5—CH_2—$
K or IV ...	... $CH_3—(CH_2)_6—$.
X or III ...	... *p*-$HO—C_6H_4—CH_2—$

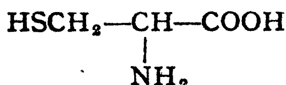
At present, the mould *penicillin chrysogenum* is used to produce the required antibiotic. The commercial methods use three different procedures : (*i*) the surface culture method, (*ii*) the submerged method and (*iii*) the bran method. It has been found that addition of phenyl acetic acid to the growth medium, increases the yield of penicillin G.

STRUCTURES OF THE PENICILLINS. The penicillins have the general structure $C_9H_{11}O_4N_2SR$. They form mono sodio salts and hence are monobasic acids; on acid hydrolysis, they give rise to (*i*) penicillamine and (*ii*) penillo-aldehyde. All the penicillins give the same penicillamine but the penillo-aldehyde differs from penicillin to penicillin (*R* varies)

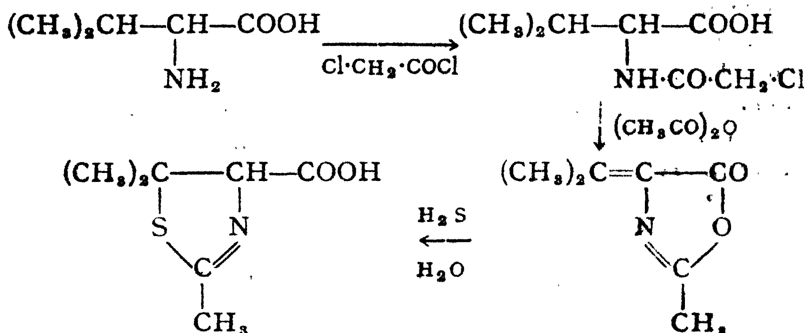


$$C_9H_{11}O_4N_2SR + 2H_2O \longrightarrow CO_2 + C_5H_{11}O_2NS + C_3H_4O_2NR$$

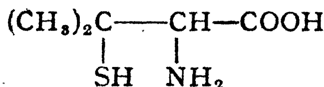
PENICILLAMINE: Its molecular composition is $C_5H_{11}O_2NS$; it gives the indigo colour reaction with $FeCl_3$. This is characteristic of cysteine.



Hence penicillamine is a simple derivative of cysteine; that it is a dimethyl cysteine is proved by a straightforward synthesis.



The latter on boiling with *HCl* and treatment with pyridine gives penicillamine



The racemic form was resolved by means of brucine, after formylation. The *D* isomer was found to be identical with the one obtained from penicillins.

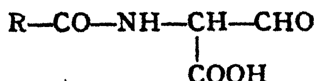
PENILLO ALDEHYDES: They have the general formula $C_3H_4O_2NR$; on hydrolysis, they give amino aldehyde and an acid *R—COOH*



Hence penillo aldehydes are to be represented by

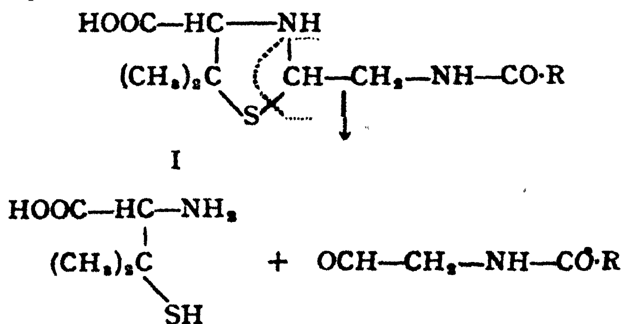


The formation of the penillo aldehydes from penicillins is accompanied by the evolution of CO_2, hence it is believed that these compounds are formed from the penaldic acids:

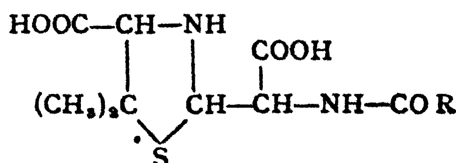


which are diketo acids capable of losing CO_2 readily.

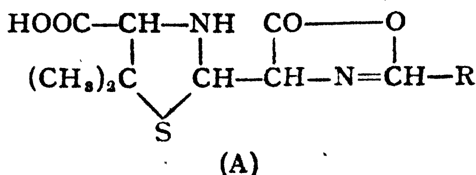
THE MODE OF LINKING OF THE ABOVE TWO UNITS IN PENICILLIN: This is established by the following evidence: mild alkaline hydrolysis of penicillin gives penicilloic acid–a dicarboxylic acid–which is readily changed with evolution of CO_2 into penilloic acid. The latter on hydrolysis with $HgCl_2$ (aqueous) gives penicillamine and penillo aldehyde. Such a decomposition is characteristic of thc azolidine ring. Hence penilloic acid must be I which accounts for the observed hydrolysis



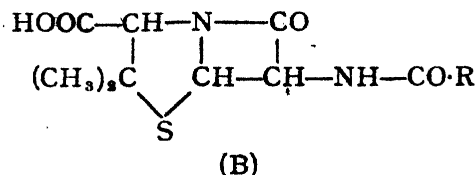
Penicilloic is a β-diketonic acid related to penilloic acid. Therefore it must be represented by the formula II.



But penicillin is a mono carboxylic acid and carries a free COOH on the thiazolidine nuecleus; because, on methylation with CH_2N_2 the methylester formed, on hydrolysis with aqueous $HgCl_2$ solution, gives the methyl ester of penicillamine. Hence the other COOH group in penicilloic acid is present as either a *lactone* structure (A) or a lactam structure (B).



(A)

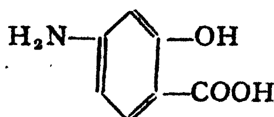


(B)

No chemical evidence has been brought forward to make a choice between these two alternative formulas. However, the x-ray analysis of the alkali salts of penicillin Gt, showed the presence of β lactam ring. Therefore penicillins have been assigned the structure (B). The penicillin Gt, the commonly used penicillin has $R \equiv C_6H_5-CH_2-$

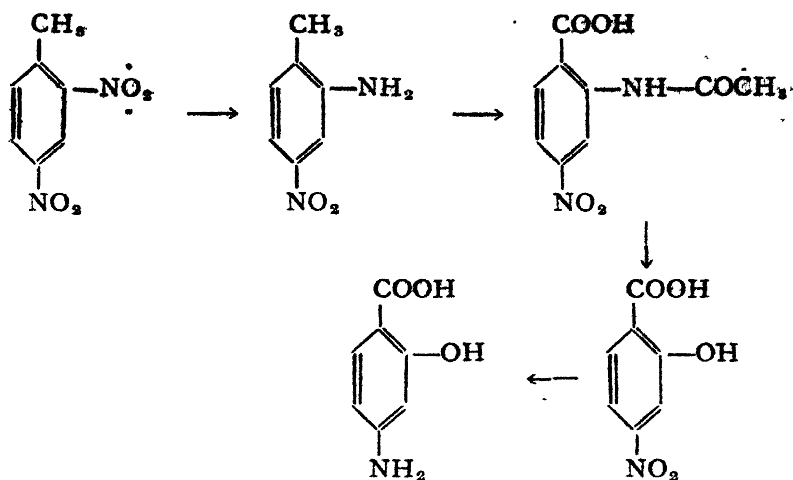
Two simple compounds which have been used in the treatment of tuberculosis are *p*-amino salicylic acid (P. A. S.) and the hydrazide of isonicotinic acid (Iso-niazid). These drugs are used often in combination with streptomycin to produce better results and to lessen the development of resistance.

p-AMINO-SALICYLIC ACID :—It is the 4 amino-salicylic acid :

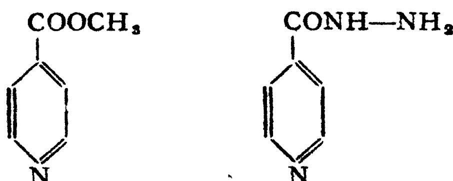


It is obtained by the following methods :

(i) Carbonation of m-nitro phenol and subsequent reduction
(ii) from 2, 4 dinitro toluene



ISO HYDRAZIDE :—Isonicotinic acid is converted into an ester and the ester with 85% NH_2—NH_2 to give the hydrazide.



In one of the recent methods, isonictinic acid and hydrazine are heated together under reduced pressure to give the hydrazide.

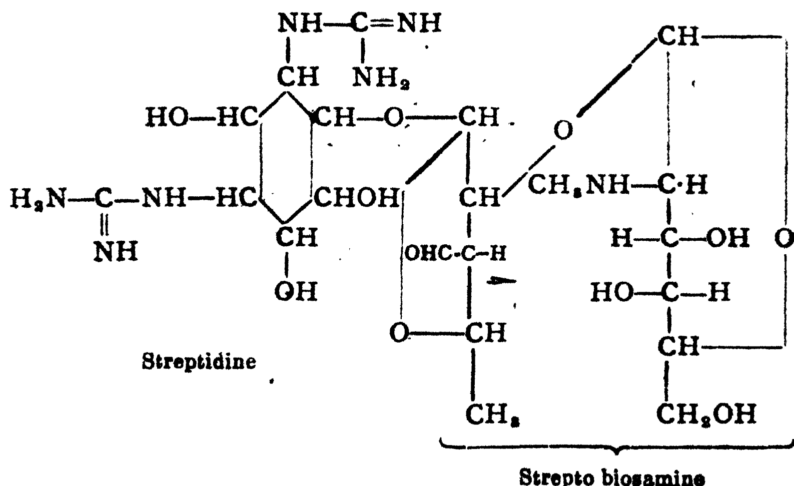
Penicillin G is characterised by its remarkably low toxicity. It is active only against Gram-positive organisms and against spirochætes. However, it loses its activity when given orally and hence has to be administered intravenously. The great disadvantage of penicillin therapy is that it is rapidly excreted. This necessitates repetition of dosages at relatively short intervals. This difficulty has been overcome in two ways : In the first method, an agent which is not toxic but which depresses the rate of elimination is used in combination with penicillin. Such an agent is 4′ carboxy-phenyl -methane-sulphon anilide : (carinamide) and has the structure :



In the other method, penicillin is converted into the salts with the local anæsthetics like procaine and benzocaine or with the alkaloid ephedrine. Thus the crystalline salt of penicillin with procaine is largely used in modern therapy. It is less rapidly eliminated, and thus greatly prolongs the action of penicillin. It also helps to minimise the pain produced at the site of the injection.

STREPTOMYCIN :—This is an antibiotic from *actiomycetes* isolated by Waksman. It is obtained by growing *streptomyces griseus* in a weakly alkaline medium by one of the three procedures used in penicillin production. The basic antibiotic is concentrated from the culture broths by adsorption and subsequent elution at the *pH* range 3–7. Outside this range, the solutions lose their activity very quickly.

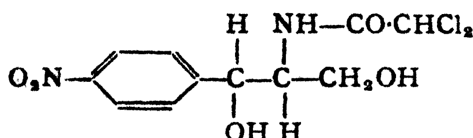
Streptomycin is a complex molecule and on hydrolysis under suitable conditions, gives (*i*) a bioside: strepto-biosamine and (*ii*) streptidine—a diguanidino-derivative. The following structure based on experimental evidence, has been proposed :



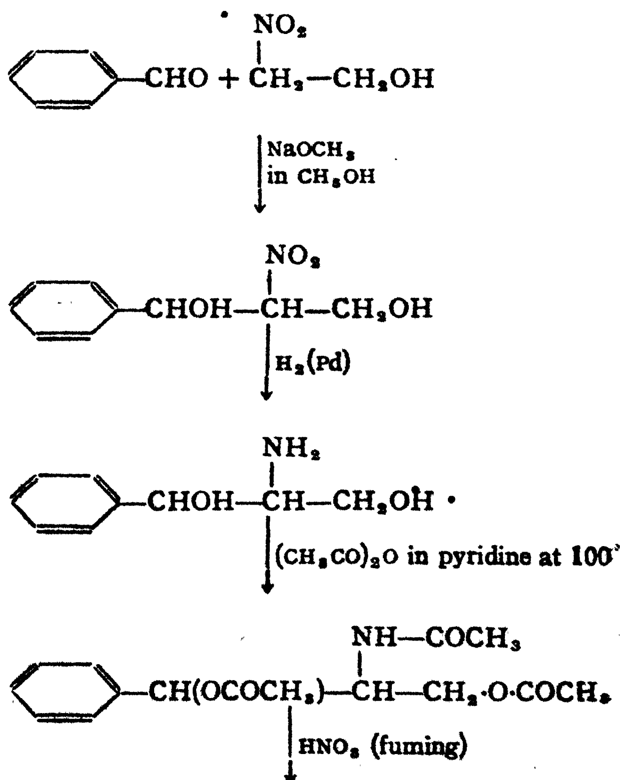
It possesses low toxicity and is active against tubercular bacteria, Dihydrostreptomycin obtained by the catalytic reduction of the antibiotic, contains a—CH_2OH group in place of —CHO in the parent compound. It is claimed that the dihydro-derivative is to be preferred as it causes less side-effects.

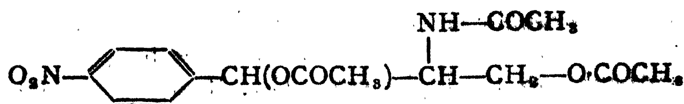
CHLOROMYCETIN or Chloramphenicol was first isolated from a *Streptomyces* species found in soils. It is now being synthesised and distributed by the Parke Davis Co. It is a chlorine bearing antibiotic. It is specifically active against thypoid and has the great advantage that it can be administered orally.

It has been assigned the following structure :



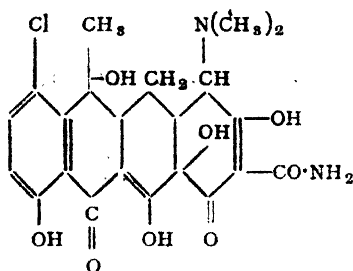
and is synthesised according to the scheme given below :—



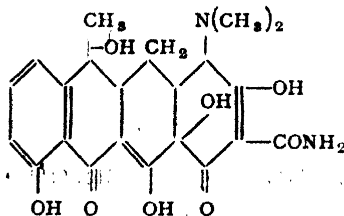


Acid hydrolysis (5 % HCl) of the above compound, was followed by resolution with *d*-damphor-sulphonic acid or with tartaric acid gives *l*-isomer. The latter, on dichloro acetylation by heating at 100° for about one hour with methyl dichloro acetate, gives a compound identical with the natural product.

Aureomycin and terramycin are the derivatives of the complex tetracycline. They have been isolated from streptomyces aureofaciens and streptomyces rimosces respectively. Both are used widely in the treatment of many diseases. The following structures have been assigned to them on the basis of extensive analytical evidence:



aureomycin

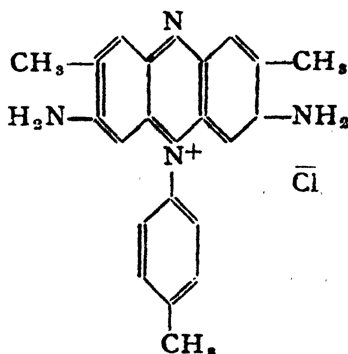


Terramycin

CHAPTER XI

SYNTHETIC DYES

Introduction:—The first synthetic dye was "*mauve*" obtained by Perkin (Sen.) in 1856, by the oxidation of commercial aniline with potassium dichromate and sulphuric acid. He obtained "a dirty, reddish, brown precipitate" from which he isolated a violet dye, mauve or mauveine. It was the product of a series of successive oxidations, and condensations. It is now known to be a *phenazine* derivative, and to belong to the *safranine* group of dyestuffs. Its formula is:—



It is a brilliant violet dye. The same year another brilliant red dye *rosaniline* was discovered. The latter belongs to the triphenylmethane class of dyes. These discoveries were followed by the preparation of many synthetic dyes. However, the development of the production of these dyes was more or less *empirical*; the structural theory of benzene was not formulated by Kekule,* and the chemical constitution of the dye was a mystery. In 1866, Kekule, developed his theory of benzene structure and in 1868, Graebe and Liebermann achieved a complete solution to the problem of the constitution of *alizarin*—the red dye from the madder root. A synthesis of the dye followed in 1869. A still greater triumph was that of Baeyer (1882). He elucidated the structure and achieved an unambiguous synthesis of the natural blue dye, *indigo*. These results greatly stimulated synthetic researches

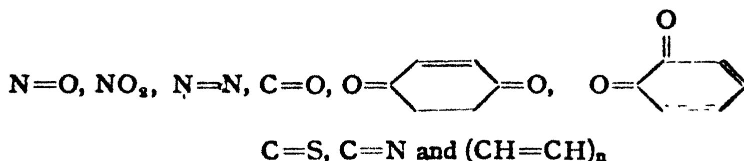
in the field of dyestuffs and paved the way for a systematic and scientific development of a synthetic dye industry.

The source-materials for the production of these dyes are the *coal-tar crudes*: benzene, toluene, phenol, naphthalene, anthracene etc. The latter are converted into *dye-intermediates* by simple reactions and procedures which include (*a*) nitration, (*b*) reduction (*c*) sulphonation, (*d*) alkaline fusion, (*e*) alkylation, (*f*) halogenation (*g*) oxidation and (*h*) condensation. The dyestuffs are then obtained by further suitable treatment of the intermediates. At present, the manufacture of the synthetic dyestuffs is not purely empirical; it has followed certain broad generalisations of relationships that have been found to exist between the colour and the chemical constitution of the coloured compound.

THE CHROMOPHORE THEORY:—A study of the constitution of organic compounds that are coloured and are used as dyes, has revealed a relationship between the structure of the molecule and its colour. This relationship is embodied in the theory of colour and dyes, first proposed by O. Witt. The essential features of the theory are:—

(*i*) Coloured substances are called '*chromogens*' (they are not necessarily dyes).

(*ii*) The chromogens owe their colour to certain *unsaturated* groups called '*chromophores*'. The most typical and common chromophores are:



$N{=}O$, NO_2, $N{=}N$, $C{=}O$, $C{=}S$, $C{=}N$ and $(CH{=}CH)_n$

The chromophoric groups are all unsaturated and hence contain mobile electrons. According to the modern theory of resonance, the mobile electrons of the chromophores are capable of being transferred to energy rich states, by absorption of radiation, thus producing colour; the auxochromes are groups which make resonance possible and hence tend to intensify the colour. The

chromophore groups differ among themselves in their power to develop colour. The observed order is :



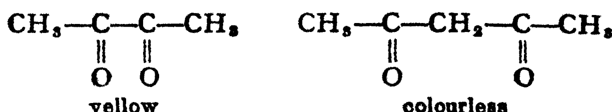
$$C=C < C=N < C=O < N=N < C=S$$

Usually, more than one chromophoric group is necessary to produce visible colour. Thus we have the series :



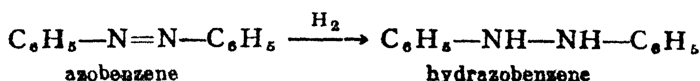
$CH_3—CO\ CH_3$ colourless $CH_3—CO{\cdot}CO\ CH_3$ yellow $CH_3{\cdot}CO{\cdot}CO{\cdot}CO{\cdot}CH_3$ yellow-orange

Further if the chromophores are conjugated the shade becomes deeper owing to relatively greater absorption.



The colour of the carotenoids is due to the presence of a large number of conjugated $—CH=CH—$ groupings.

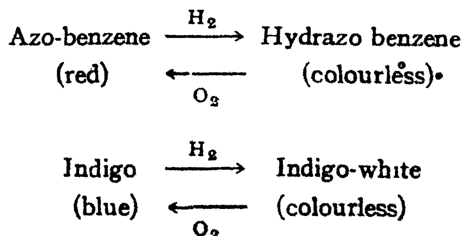
Azobenzene, anthraquinone, dinitro-benzene are chromogens being coloured due to the presence of $N=N$, $CO—CO$, NO_2 groups respectively. The chromogens, on reduction, give colourless compounds *e. g.*, azo-benzene, a bright red compound, on reduction, forms the colourless hydrazo-benzene :—



$$C_6H_5—N=N—C_6H_5 \xrightarrow{H_2} C_6H_5—NH—NH—C_6H_5$$

azobenzene hydrazobenzene

Very often, the conversions are reversible; whenever such is the case, the reduction products are called "*leuco*" compounds :—

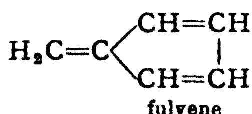


(Sometimes reduction completely decomposes the coloured compounds; such reduction products are not called *leuco* compounds).

In the case of the anthraquinone dyestuffs, the leuco compounds are coloured; but the shade that appears on the dyed fabric is different from the colour of the reduced compound *i. e.* the vat.

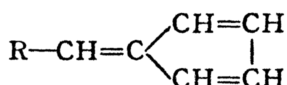
Closely related to the leuco compounds are the bisulphite compounds derived from dyestuffs containing CO groups, by treatment with a solution of $NaHSO_3$. These compounds are soluble in water (even though the original dyestuffs are insoluble) and are readily reconverted into the parent substance, on steaming or on treatment with alkalies. Alizalrin Blue S is the bisulphite compound of Alizarin Blue.

Compactness of the atoms in the molecule also appears to cause the development of colour. Open chain compounds are rarely coloured, while all the organic coloured molecules are derived from *closed* systems. Fulvene, isomeric with benzene but more compact, is a distinctly coloured compound.



fulvene

Similarly, cyclopentadiene condenses with *R—CHO* to give highly coloured fulgides :—



(*iii*) A dye is a chromogen which contains, in addition to the chromophore, a second group, called '*auxochrome*'. Such a group is a salt-forming group, *i. e.*, *basic* or *acidic* and makes the coloured compound attach itself to the fabric in such a way that it is fast to light, soap and water. The auxochrome is also a solubilizing group and tends to intensify the shade. The auxochromes are classified as (*i*) acidic; OH, COOH, and $—SO_3H$ and (*ii*) basic: NH_2, NHR and NR_2; the former give rise to acidic dyes and the latter, to basic dyes. The relative order of the colour-intensifying effect of the auxochromes is:



$$NR_2, > NH{\cdot}R, > —NH_2, > —OH, > —OCH_3$$

Acetylation of the NH_2 and OH group considerably decreases their auxochromic effect. The halogen atoms also function as auxochromes: the relative order is I > Br > Cl. It is obvious that all the auxochromic groups carry atoms, with unshared electron pairs; they thus exert their influence when linked to chromophore groups either directly or through a conjugated system. Alkyl groups on account of hyperconjugation, are also capable of influencing light absorption and thus function as auxochromes. The auxochrome *COOH* and SO_3H when introduced into a chromogen, give a dye without much alteration of the shade. Recently, Friedlander has shown that SO_3H group, causes great change in the shade of indigo, when present in certain positions. Usually, the position of the auxochrome in the dye molecule has a decided influence on the tinctorial properties and the shade of the dye.

From the foregoing considerations, it follows that a dye molecule must necessarily contain a chromophore group and an auxochrome group. Azobenzene $C_6H_5—N=N—C_6H_5$ is a chromogen containing the chromophore, N=N; it is a bright red compound, but is not a dye. On the other hand, *p*-hydroxy-azo benzene *p*-$HO—C_6H_4—N=N—C_6H_5$ and *p*-amino azo benzene, *p*-$H_2N—C_6H_4—N=N—C_6H_5$ are both dyes, as they contain the auxochromes —OH and —NH_2 respectively. The former is an acid dye and the latter is a basic dye.

Classification of the Dyes—The actual number of dyes known is enormous. They have been classified according to :

(*i*) Their mode of application to the fabric; thus, we have, (*a*) Substantive or direct dyes that can be directly applied to the fabric. (*b*) Adjective or mordant dyes that require the use of a mordant. (*c*) Vat dyes in which case, the dyeing is carried out in vats. (*d*) Ingrain dyes, etc.

(*ii*) The nature of the auxochrome present: this gives us the division of the dyes into (*a*) acidic and (*b*) basic dyes.

(*iii*) The constitution of the dye as defined by the type of the

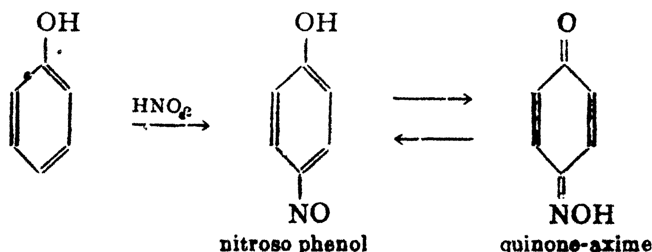
chromophore contained in the dye molecule. According to this, the following are some of the important classes of dyestuffs :—

(1) Nitroso and Nitro-dyestuffs
(2) Azo-dyestuffs
(3) Triphenyl-methane dyestuffs
(4) Anthraquinone dyestuffs.
(5) Quinoneimine dyestuffs which include indamines, oxazines, azines, and thiazines.
(6) Indigoids
(7) Acridine dyestuffs
(8) Pyrazolone dyestuffs
(9) Sulphur dyestuffs.

A more systematic classification will be the one in which, the structural units provide a basis for division and the mode of application, a basis for subdivision. Thus the dyestuffs containing the chromophore N=N are grouped under azo dyes and may be further subdivided into (*a*) Adjective dyes, (*b*) Basic dyes, (*c*) Acidic dyes etc. The Society of Dyers and Colorists have adopted a classification based on constitution. This classification given in the British Colour Index (Rowe) is accepted both in U. S. A. and in England; according to this, the dyes are classified into 26 types.

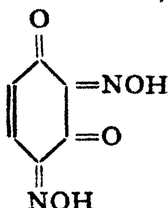
Nitroso and Nitro Dyes

NITROSO DYES :—They are obtained from phenols and naphthols by nitrosation *i. e.* action of nitrous acid in the cold. Structurally, they are tautomeric with the quinone–oximes.



O–nitrosophenols form metal complexes and hence are used as 'mordant dyes; with iron mordant, they give intensely green shades. 2, 4 Dinotro-resorcinol is a typical nitroso dye. It is obtained by

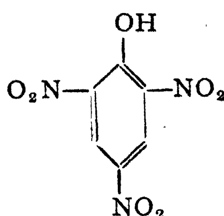
the addition of dilute H_2SO_4 to an ice-cold mixture of resorcinol and $NaNO_2$ in water. It has the structure:



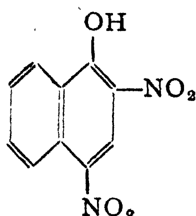
It gives olive green shades on iron mordants.

Nitrosation of the naphthols gives the corresponding nitrosonaphthols; 2 nitroso 1-naphthol is obtained from 1-naphthol and possesses mordant-dyeing properties. 1 nitroso-2-naphthol is prepared by the addition of $NaNO_2$ to a cooled solution of β-naphthol in alkali and subsequent acidification with dilute H_2SO_4. It has been used as a dye for the purposes of camouflaging in war, as it gives shades of green (Fe), yellow (Zn) and brown (Cr) resembling the shades in nature. It finds some application as a reagent for the detection and estimation of cobalt in presence of nickel. 1–Nitroso–2–naphthol–6 sulphonic acid obtained by the nitrosation of Schaffer's acid is much more useful. With iron mordants it is used to dye wool a fast green. It is also used for the acceleration of solar evaporation of sea-water for the manufacture of common salt and potassium chloride.

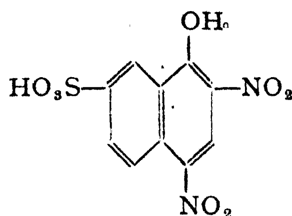
NITRO DYESTUFFS :—The majority of these dyes are nitro derivatives of phenols, naphthols or their sulphonic acid derivatives. The mono-nitrophenols give coloured salts but are not dyes. However, the tinctorial properties are developed with the di-or-tri-nitro derivatives. The relative positions of OH and NO_2 groups have a decided influence; the *ortho* and *para* positions are found to be the most favourable ones. The tinctorial property of the dye increases with the acidity of the molecule. The acidity of NO_2 group may be enhanced by the auxochromes like OH, $COOH$ and SO_3H, and also by increasing the number of NO_2 groups. Hence, some of the typical dyestuffs belonging to his group carry these auxochromes. Thus we have:



Picric acid

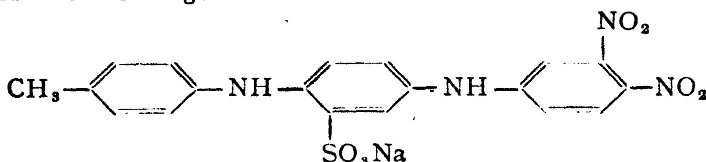


Martius yellow



Naphthol yellow

All these dyes dye the fabric yellow. Naphthol yellow is also used as a food colour. Faster dyes of this group are derived from Diphenylamine and are those which contain several—NH—Ar and—NH—linkings.



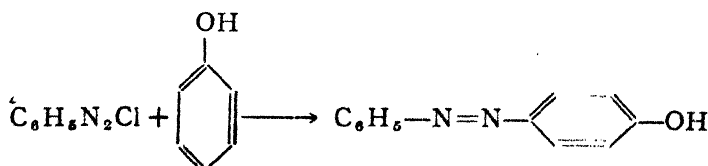
Amido yellow E.

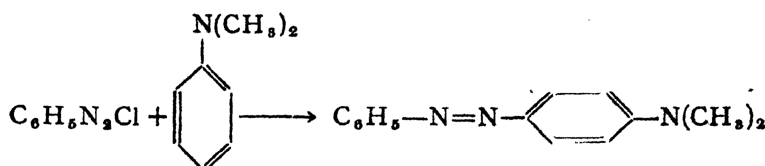
A few of the nitro dyes find use as pigments. Nitro groups are often introduced into other types of synthetic dyes, in order to modify and improve the dyeing and fastness properties of the dye molecule.

Azo Dyes

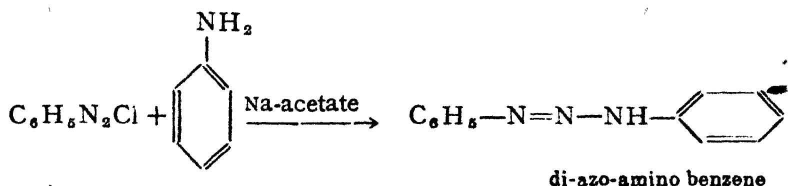
This is one of the largest and most important class of dyes. Structurally, they are hydroxy or amino-substituted azo-benzenes; the chromophore, is, thus,—$N=N$— group, and we have, (*i*) *monoazo,* (*ii*) *bis-azo* and (*iii*) *tris-azo* dyes containing respectively one, two or three azo groups. They have been further classified into: (*a*) acid dyes with OH, SO_3H as auxochromes, (*b*) basic dyes with NH_2, $NHCH_3$ and $N(CH_3)_2$ as auxochromes.

The azo dyes are obtained by "*coupling*" of a diazonium salt with a phenol or an amine in alkaline medium (*p*H5-9) or acid medium (*p*H3·5-7) respectively :—





With aniline and other unreactive primary amines, diazo-amino-compounds are formed :



di-azo-amino benzene

On the other hand, with reactive primary amines *e. g.*, α and β naphthylamines, *m*-toluidine, *m*-phenylene diamine, the azo-dyes are obtained. The coupling takes place in *para* position to *OH* or NH_2 or $N(R)_2$ group ; if the *para* position is blocked, the coupling takes place in the *ortho* position. If both the positions are not free, coupling may not take place unless the azo-group displaces the substituent in para position ($COOH$, SO_3H are sometimes displaced)



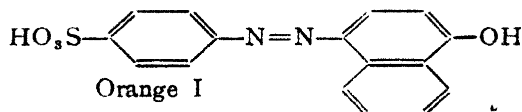
In the case of β naphthol, the coupling takes place in position 1 only ; if this position is blocked, no coupling takes place.

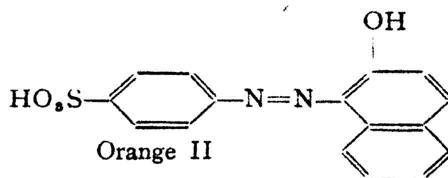
For the preparation of a poly-azo-dyestuff, the amino group of a mono-azo-dye is diazotised and the product coupled with an aromatic amine to give a dis-azo-dye. The amino group of the latter may be subsequently diazotised and coupled with another amine to give a triazo compound and the process may be repeated.

The simplest azo-dyes are yellow : the introduction of alkyl phenyl groups or an increase in the molecular weight changes their colour through orange and red to violet. With an increase in the number of azo-groups also, the shade is deepened, but the coupling becomes more difficult; a diminished affinity for the fibres also appears in the case of the poly-azo-dyestuffs.

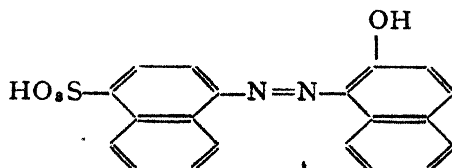
The dyes are crystalline and insoluble in water but soluble in alcohol. They are very often employed in the form of sulphonic acid derivatives. Most of the common azo-dyes are thus *acid* dyes with the SO_3H group and are *adjective* dyes or *mordant* dyes. They cannot be applied to the fabric without the use of mordant. The mordant is a substance which helps to fix a dye to a fabric which otherwise whould not take up the dyes. There are: *(a) acid* mordants like tannic acid, stannic chloride, etc. which are used with basic dyes; and *(b)* basic mordants *e. g.* metallic hydroxides which are used with acid dyes. Usually, basic salts or the acetates like ferric acetate, chromium acetate and aluminium acetate which on hydrolysis, give the corresponding hydroxides, are employed.

MONO-AZO-DYES:—Some important dyes belonging to this class are: Orange I and II, Fast Red A, methyl orange and Para Red. Orange I and II are obtained by coupling diazotised sulphanilic acid with α and β naphthols respectively under suitable conditions:—

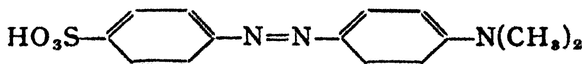




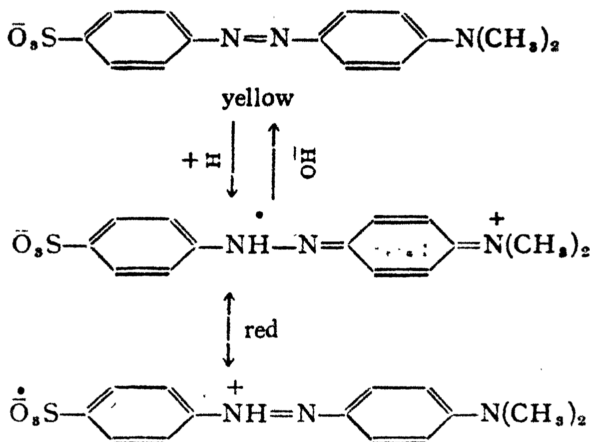
Orange II is faster than Orange I and is still used; if instead of sulphanilic acid, its naphthalene analogue, naphthionic acid is used, the well-known dye Fast Red A is obtained.



Methyl orange is obtained by coupling diazotised sulphanilic acid with dimethylaniline dissolved in HCl and adding Na-acetate.

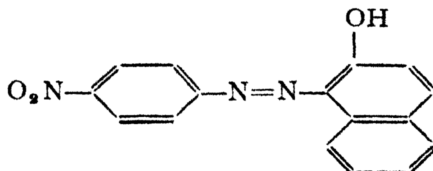


It is not a dye, the Na-salt is used as the indicator in acidimetry and alkalimetry as its colour change is sharp over a small pH range.

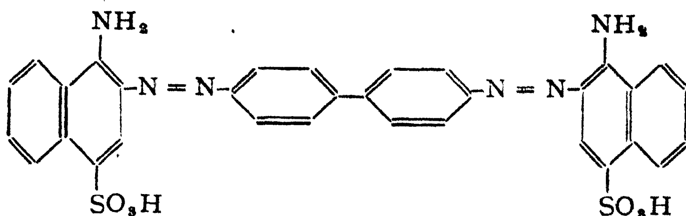


Thus in acid solutions, two resonance structures are possible and hence the solution is red.

Para Red is a common red dye and is obtained by coupling diazotised *p*-nitroaniline with β naphthol in 10% aqueous alkali.



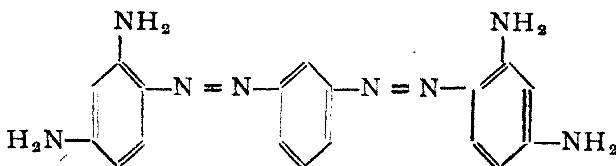
BIS-AZO-DYES :—The two typical dyes are Congo Red and Bismark Brown. Congo Red is obtained by coupling tetrazotised benzidine with naphthionic acid.



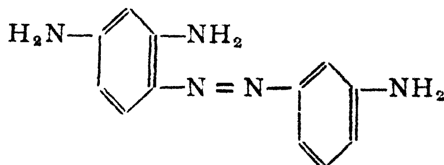
It is used as a direct or substantive dye, which can be applied to the fabric without the aid of a mordant. It is also employed as an indicator and turns *blue* in acid and *red* in alkaline medium.

The dyes derived from benzidine and substituted benzidines are of great technical value as they are substantive dyes. Benzidines containing substituents *ortho* to the NH_2 group yield substantive dyes whereas the corresponding *meta* derivatives give dyes with very little affinity for cotton.

Bismarck brown :—It is obtained by the action of nitrous acid on *m*-phenylene-diamine; one molecule is tetra-azotised which then couples with two other molecules of the same to give :—



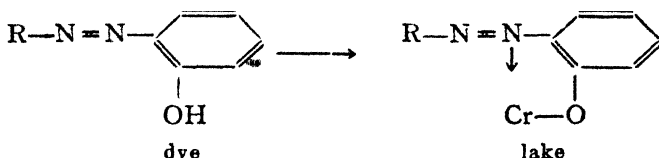
It is probable that a small amount of monoazodye :



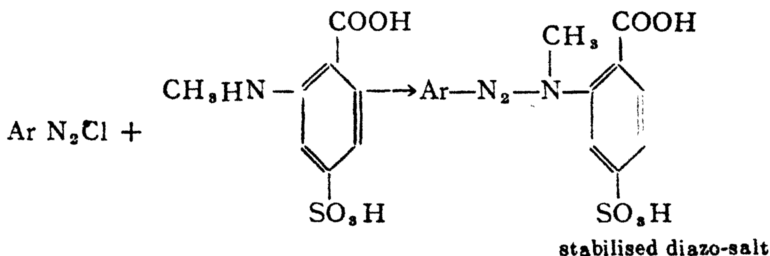
is also present. It is much used in the dyeing of leather.

Chrome Developed Azo-Dyes :—Wool is dyed with an azo-dye in the usual way and then treated with a solution of potassium dichromate; the shade suffers a remarkable change; it darkens and becomes very fast. Very resistant black shades have thus been obtained. They are termed the chrome developed azo-dyes.

The azo-dye in this case is derived from *o*-amino-phenol and is partially oxidised by potassium dichromate. The trivalent *Cr*... then forms a *lake* which is probably a chelate compound:—



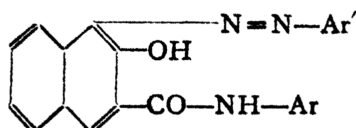
THE NAPHTHOL AS COLOURS:—Structurally, they are azo-dyes, obtained by coupling diazotised amines with aryl amides of 2-hydroxy-3-naphthoic acid. They are also called the ingrain or developed dyes; the making of these dyes is based on the following principle. The diazo salt is converted into the diazoimino compounds of secondary amines, carrying SO_3H or $COOH$ groups; the resulting compounds are soluble in water or weak alkali.



In this form they are suitable for calico printing. They are dissolved with naphthol derivatives with which no coupling occurs under alkaline conditions. The thickened solution is printed on calico and dried without change. When the print is acidified, the diazo compound is regenerated and at once couples with the aryl-amide which is finely divided and in intimate contact with the diazo-salt. An insoluble pigment is thus formed in the fibre. The acid treatment is usually applied either by immersing the print in a hot solution of Na_2SO_4, acidified with acetic acid and formic acid, or by aging with steam carrying acetic acid vapour.

Thus this method of dyeing is based on the principle of delayed coupling. A whole series of dyes have thus been prepared and they

are called "Rapidogens." Structurally, they are represented by the general formula:



(Ar and Ar′ may be a simple or complex aryl residue).

There is another way, in which stable diazo salts are produced. They are treated with neutral sulphite solutions, to give diazosulphonates which are stable under alkaline conditions, so that they can be incorporated into printing pastes with other coupling components. To obtain full development of the azo-colour, a neutral oxiding agent is necessary; the most commonly used one, is alkali chromate. Such dyestuffs are called "rapidozols."

The technique of delayed coupling has also made possible, an improved method of a new photographic process called Ozalid process.

CONSTITUTION OF AN AZO-DYE:—The azo-dye, on reduction with tin and hydrochloric acid, gives a mixture of amines. Thus we have:—



$$Ar—N=N—Ar' \longrightarrow ArNH_2 + Ar'NH_2$$

i.e. one NH_2 group is generated on each of the two aromatic nuclei linked by—N=N—group. The acid reduction is accompanied by the formation of a small amount of benzidine derivatives; hence, reduction is preferred with "hydros," $Na_2S_2O_4$ in alkaline conditions. The structures of the amines thus formed are next established and the constitution of the dye then deduced. Thus, if a dye *A* gives on reduction, a mixture of equimolar quantities of diamino benzene and tri-amino-benzene, it follows that the constitution of *A* is



$$H_2N—C_6H_4—N=N—C_6H_3(NH_2)_2$$

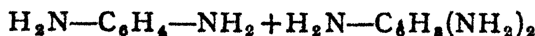
because, on reduction, it gives the abovementioned products:—



$$H_2N—C_6H_4—N=N—C_6H_3(NH_2)_2$$

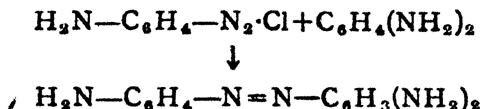


$$\downarrow H_2$$



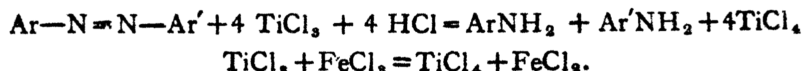
$$H_2N—C_6H_4—NH_2 + H_2N—C_6H_3(NH_2)_2$$

Such a decomposition also suggests a possible synthesis of (*A*). It can, thus, be synthesised by diazotising a molecule of $C_6H_4(NH_2)_2$ and coupling it with a second molecule of the same :—



$$H_2N—C_6H_4—N_2·Cl+C_6H_4(NH_2)_2 \downarrow H_2N—C_6H_4—N=N—C_6H_3(NH_2)_2$$

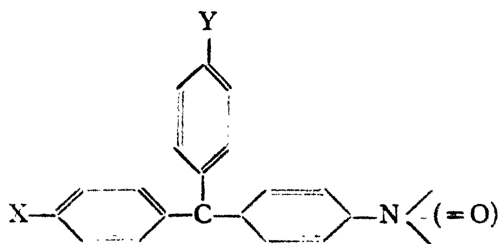
An azo-dye is estimated by titration with a standard solution of $TiCl_3$ solution. The dye is reduced with an excess of $TiCl_3$ solution in CO_2 atmosphere, and the excess titrated against standard $FeCl_3$ solution with KCNS as the indicator.



$$Ar—N=N—Ar'+4\ TiCl_3 + 4\ HCl = ArNH_2 + Ar'NH_2+4TiCl_4$$
$$TiCl_3+FeCl_3=TiCl_4+FeCl_2.$$

Triphenyl-Methane Dyes

The first synthetic dyes, rosaniline and para-rosaniline belong to this class of dyes. They all contain *three* phenyl groups (hence, the name) and possess the following structure :—



Various groups may be present in the benzene rings, but the essential conditions for the formation of a dye are :—

(*i*) at least *two* NH_2 or *OH* groups in positions X and Y (they are the auxochromes), must be present.

(*ii*) At least one of the three benzene rings must be quinonoid in structure.

It is the above grouping that constitutes the chromophore of this class of dyes. They possess a brilliant shade, are red, violet, blue or green. But they are not fast to light; several are sensitive to the action of alkali or to soaping. They are relatively cheap.

The triphenyl methane dyes have been subdivided into three main groups, on the basis of the nature of the auxochromes; thus we have :—

(i) The fuchsine group :—rosaniline, pararosaniline, malachite green.

(ii) The aurin group :—aurin and rosolic acid.

(iii) The phthalein group which has been further subdivided into *(a)* eosins and *(b)* rhodamines. The phthaleins are also referred to as pyronines.

The fuchsine group of dyes contains NH_2, NHR or $N(R)_2$ groups as the auxochromes; the aurin group contains OH group as the auxochromes and the phthaleins are derived from phthalic anhydride and phenolic compounds and contain OH and $COOH$ groups as auxochromes as in the eosins and $N(R)_2$ and $COOH$ groups as auxochromes, as in the rhodamines. The auxochromes must be present in *para* or *ortho* positions. The meta-amino derivatives of triphenyl-methane exhibit no tinctorial properties.

Relation of the Dyes to Triphenyl-methane :—The dyes, rosaniline and para-rosaniline, form the typical members of this class of dyes. Their relationship to triphenyl-methane $(C_6H_5)_3 \cdot CH$ was definitely established by the brilliant researches of Otto and Emil Fischer.

Para-rosaniline was prepared from a mixture of aniline and *p*-toluidine by oxidation, and had the molecular composition $C_{19}H_{18}N_3Cl$. It gives the following reactions :—

(a) On reduction with zinc and acetic acid, it gave a colourless compound $C_{19}H_{19}N_3$.

(*b*) The dye was diazotised and on warming, a tri-hydroxy compound was obtained. These results indicated the presence of *three amino* groups.

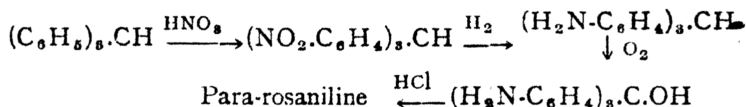
(*c*) The diazonium salt, on boiling with alcohol, was converted into a hydrocarbon, $C_{19}H_{16}$ (The diazonium group was replaced by hydrogen). As the starting-point for the preparation of the dye was a mixture of aromatic amines, the presence of phenyl radicals was

indicated and the hydrocarbon was formulated as $(C_6H_5)_3\ CH$. It was later on, identified as *triphenyl-methane* synthesised from chloroform and benzene:—



$$CHCl_3 + 3C_6H_6 \xrightarrow{AlCl_3} CH(C_6H_5)_3$$

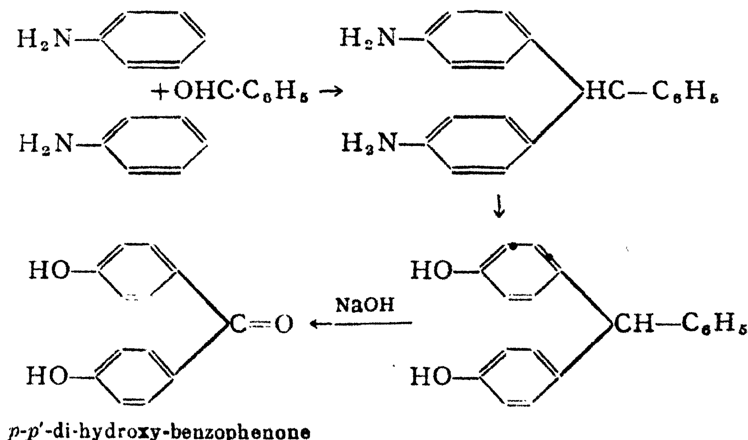
Thus, the dye para-rosaniline was shown to be a derivative of $(C_6H_5)_3CH$. The exact relationship of the dye to this hydrocarbon was then established by a direct synthesis of the dye from triphenyl-methane itself. The various steps involved were:—



$$(C_6H_5)_3.CH \xrightarrow{HNO_3} (NO_2.C_6H_4)_3.CH \xrightarrow{H_2} (H_2N\text{-}C_6H_4)_3.CH \xrightarrow{O_2} (H_2N\text{-}C_6H_4)_3.C.OH \xrightarrow{HCl} \text{Para-rosaniline}$$

The positions of the amino groups were settled by the following evidence :—

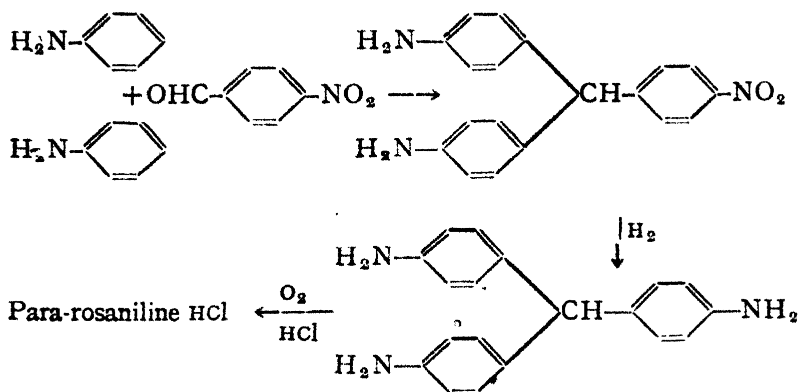
The tri-hydroxy compound obtained by diazotisation and subsequent warming yield on oxidation, *p*-hydroxy-benzoic acid. Further, benzaldehyde condenses with aniline to give a diamino-triphenyl-methane derivative which can be converted into a di-hydroxy derivative. On fusion with alkali, the latter forms di-hydroxy benzophenone :—



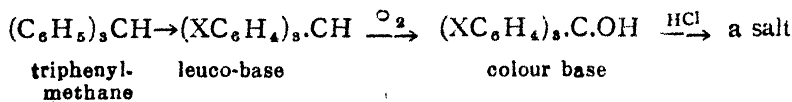
p-*p*'-di-hydroxy-benzophenone

(The structure of the di-hydroxy-benzophenone follows from its synthesis from *p*-hydroxy-benzoic acid).

These results, thus, indicate that the two *OH* groups and hence the two NH_2 groups are in *para* positions to the methane carbon atom. The position of the third NH_2 group is proved as follows :— Aniline is condensed with *p*-nitro-benzaldehyde and subsequently converted into para-rosaniline :—



The dyes are *salts* formed by the action of acids on certain derivatives of *triphenyl carbinol*, which are called *colour* bases. The latter are formed by the oxidation of *leuco* bases which are simple derivatives of tri-phenyl-methane. When the colour base is converted into a salt, quinonoid structure is developed and the product is a coloured dye :—



$$(C_6H_5)_3CH \rightarrow (XC_6H_4)_3.CH \xrightarrow{O_2} (XC_6H_4)_3.C.OH \xrightarrow{HCl} \text{a salt}$$

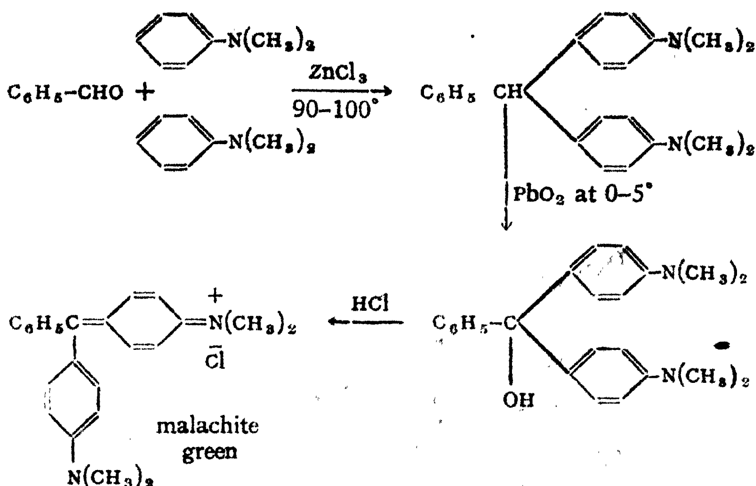
triphenyl-methane leuco-base colour base

$X = NH_2$, OH, or—SO_3H.

Fuchsine Group

These dyes are basic dyes and give bright shades. On sulphonation, they yield dyes which are acid dyes. The most important and common dyes of this class are malachite green, rosaniline, pararosaniline, methyl violet and crystal violet.

Malachite green :—This is obtained from benzaldehyde and dimethylaniline according to the following scheme :—

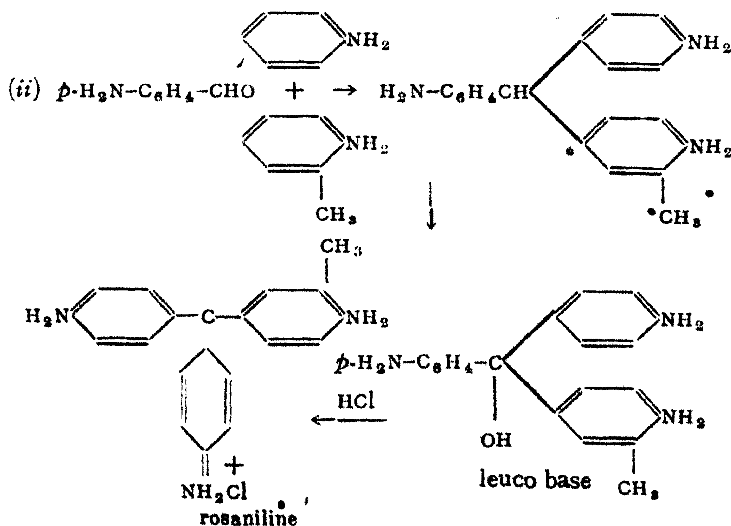


commercially, it is used in the form of its crystalline oxalate or the double salt with zinc chloride.

Rosaniline :—This dye is obtained by oxidising a mixture of aniline, *o*-toluidine and *p*-toluidine with mild oxidising agents like $C_6H_5NO_2$ or arsenic acid. The carbon atom of the methyl group of the *p*-toluidine furnishes the methane carbon atom for the linking of the phenyl radicals. The chemical changes involved are :—

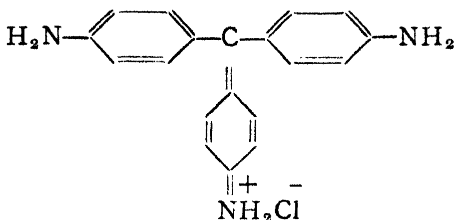


(*i*) *p*-$H_2N-C_6H_4-CH_3 \rightarrow$ *p*-$H_2N-C_6H_4-CHO$.



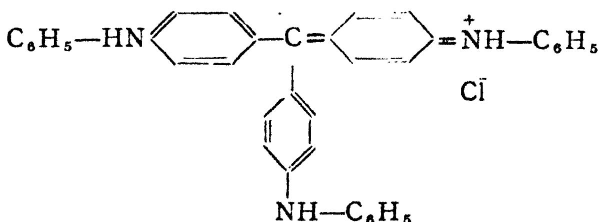
Stannic chloride and mercuric nitrate can also be used as the oxidising agents. Nitrobenzene has now superseded all other oxidising agents.

Para-rosaniline :—It is prepared by oxidising a mixture of aniline and *p*–toluidine with $C_6H_5NO_2$, and possesses the structure.



Methyl violet and crystal violet are extensively used as antiseptic in modern medical practice; they have been discussed earlier under "Synthetic drugs."

Aniline blue :—This is an *N*-aryl derivative of para-rosaniline. It is obtained by heating the latter with aniline in presence of acetic acid or benzoic acid; arylation takes place and three phenyl groups, one on each *N* atom are introduced; the dye has the structure:



The dye obtained by arylation is a blue dye. Recently some of the triphenyl methane dyes *e.g.* crystal violet are obtained from the Michler's ketone.

MODIFICATION AND IMPROVEMENT OF SHADES :—The shades of these dyes are varied by the introduction of radicals in place of *H* atoms of NH_2 groups in the dye molecule.

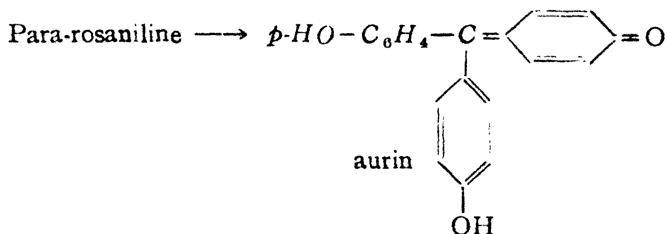
(*a*) Methylation, thus gives *violet* dyes, *Methyl violet* and crystal violet are *penta* and *hexa*-methyl derivatives of para-rosaniline.

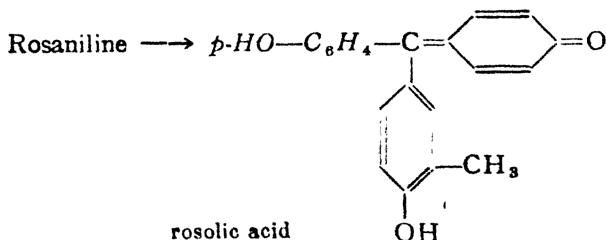
(*b*) Introduction of phenyl groups yields *blue* dyes; thus, *aniline blue* is a triphenyl derivative of para-rosaniline.

(*c*) Substitution of the nuclear *H* atoms by methyl groups has little or no effect on the colour. Thus, para-rosaniline and rosaniline (its methyl homologue) have the same shade. Similarly, introduction of *Cl*, CH_3,SO_3H,OCH_3 groups etc. into *meta* or *para* positions has a small effect on the colour. In the *ortho* positions, these substituents have the useful property of increasing the resistance of the dye to alkali. Such an effect seems to be truly steric, as the group prevents the access of the *OH* ion to the central carbon atom.

Aurin Group

The dyes of this group are the hydroxy-derivatives of triphenyl-methane. Aurin and rosolic acid are the typical members and are simply related to para-rosaniline and rosaniline respectively. Thus, they are obtained by diazotising the latter dyes and warming the diazotised solution with dilute H_2SO_4, when the NH_2 groups are replaced by *OH*.



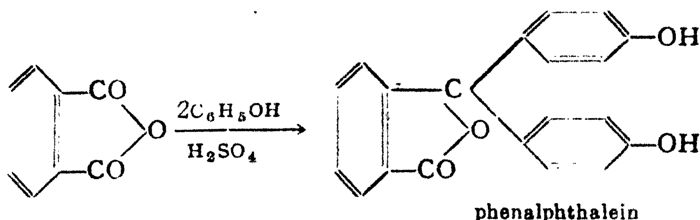


Aurin is also obtained by heating phenol with oxalic acid and concentrated H_2SO_4. Oxalic acid is first decarboxylated to formic acid which supplies the methane carbon atom. Many dyes of this class are also prepared commercially by heating formaldehyde with substituted phenols in presence of H_2SO_4 containing nitrite, *e. g.* aurin tricarboxylic acid is thus obtained from formaldehyde and salicylic acid. It is used as a mordant dye.

Phthalein Group

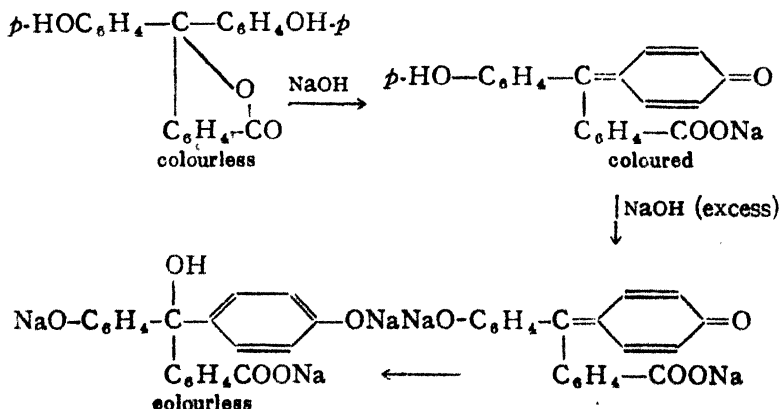
This group has been subdivided into (*a*) the eosins and (*b*) the rhodamines. Phenol-phthalein, fluorescein, gallein etc. constitute the eosins; they are the triphenylmethane dyes with OH and COOH groups as auxochromes. They are readily obtained by condensing phenols with phthalic anhydride, with or without a condensing agent like H_2SO_4, $ZnCl_2$ or $SnCl_4$. The representatives of the class are phenolphthalein, fluorescein, eosin, and the phloxins.

PHENOLPHTHALEIN: It is obtained by heating phenol with phthalic anhydride alone or in presence of $ZnCl_2$ or H_2SO_4 at 115–120°.



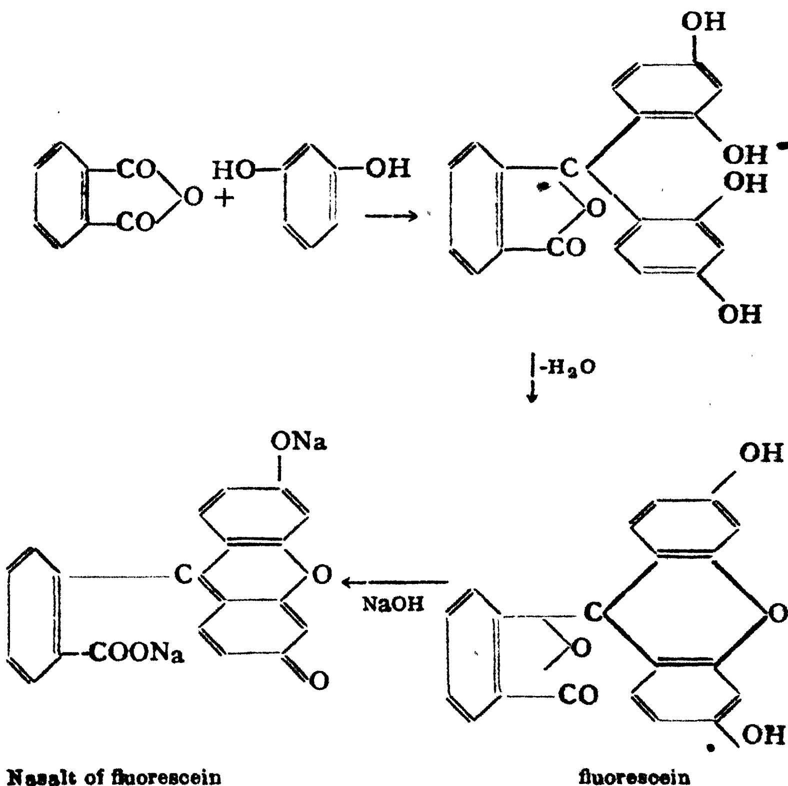
phenalphthalein

It is soluble in alkali, forming a salt which is pink. It is used as an indicator in acidimetry and alkalimetry, it is colourless in acid and pink in alkaline medium; with excess of alkali, the colour disappears. These changes are connected with structural alterations, which the molecule undergoes under the different conditions of acid and alkali. Schematically, we have :—



Eosins are formed by the condensation of *m*-dihydroxy-phenols with phthalic anhydride in presence of a suitable dehydrating agent. Fluoroscein is an important eosin.

FLUORESCEIN : It is obtained by heating phthalic anhydride with resorcinol at 200° or in presence of H_2SO_4 at 115° to 120°.

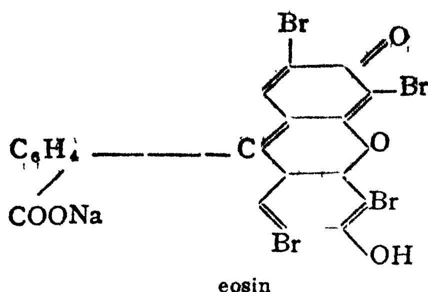


Nasalt of fluorescein

fluorescein

Fluorescein dissolves in alkali to give a beautifully green fluorescent solution. The fluorescence is so intense that its formation is used as a test for resorcinol or for phthalic anhydride.

Eosin :—It is the sodium salt of the tetra-bromo derivative of fluorescein and is obtained by the action of bromine in acetic acid on fluorescein :—

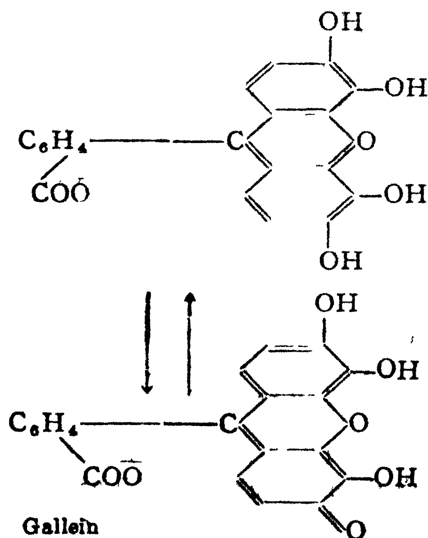


eosin

It is used as a dye for silk and wool and also in the preparation of red inks. It also finds applications as a staining material. The corresponding iodo-derivative is erythrosin. The latter is obtained by the action of iodine in KI on an alcoholic solution of fluorescein. Mercurochrome is a mercury derivative of dibromo-fluorescein used as an antiseptic.

The brightly coloured phloxines are obtained by condensing phenols with di - or tetra-chloro-phthalic anhydrides. They are used as dyes.

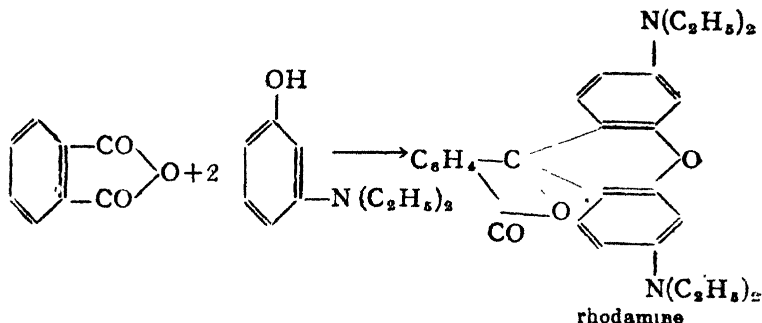
Resorcinol may be replaced by other *m*-dihydric phenolic compounds to give the corresponding fluoresceins. Thus, phthalic anhydride and pyragallol or gallic acid give *gallein.*



Gallein

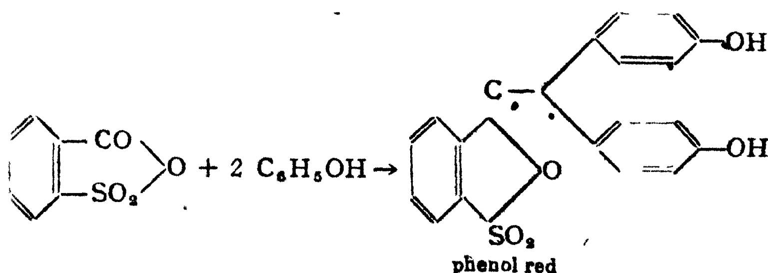
N.B.—The alkali salts or eosins can be formulated as both (*a*) orthoqinonoid or (*b*) para-quinonoid structures.

Rhodamines :—A typical member of this group of dyes is obtained as follows : phthalic anhydride is condensed with diethyl-*m*-amino-phenol at 175° in presence of anhydrous $ZnCl_2$.



rhodamine

The diethyl derivative is more valuable than the corresponding methyl derivative, on account of its greater solubility. The rhodamines are basic dyes and dye wool and silk direct in bluish-red shades with strong fluorescence. Further improvement in the shade is obtained by esterifying the COOH group as in rhodamine B. The sulphonated derivatives of the rhodamines are much faster dyes than the basic parent rhodamines and are of great value in wool dyeing. Recently new indicators of sulphophthalein group are much used in acidimetry and alkalimetry. They are formed by the condensation of phenols with o-sulpho-benzoic acid or its anhydride. Phenol sulphophthalein or phenol red, is a typical member of this class.



phenol red

It is yellow in acid solution and red in alkali. The colour changes take place within the *pH* range of 6·8 to 8·4. The colour is, thus, sharper than that of phthaleins.

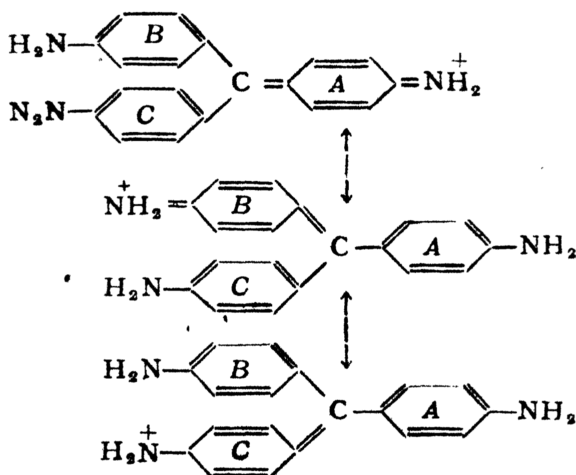
STRUCTURE OF TRIPHENYL-METHANE DYES :—Nietzki was the first to propose for para-rosaniline a structural formula in which one of the three benzene nuclei existed in the quinonoid form. It is the quininoid or quinoid grouping that is the *chromophore.* However, the above formula fails to explain some facts.

(i) The formula suggests that only one NH_2 group is necessary for the formation of the dyestuff which is not the case, because *mono*-amino-triphenyl-methane derivative yields no dyestuff.

(ii) Addition of excess of an acid to a triphenyl-methane dye like crystal violet, changes its colour through violet, green, to weak orange. This is probably due to the formation of an ammonium ion and that colour depends on the presence of $N(CH_3)_2$ groups.

(iii) These dyes (salts) possess intense absorption.

In order to account for these facts, it is now suggested that each of the three benzene nuclei in turn possesses the quinoid structure. In other words, *resonance* comes into play and the actual state of the ion is that of a resonance—hybrid between the states in which all three amino groups and all the three nuclei are equivalent :—



Further, when the amino group becomes quarternary by salt-formation, it cannot contribute to a possible resonance state. But resonance demands two or more possible alternative states. Hence

the diamino, and triamino derivatives constitute dyestuffs, while the mono-amino compound possesses poor dyestuff properties.

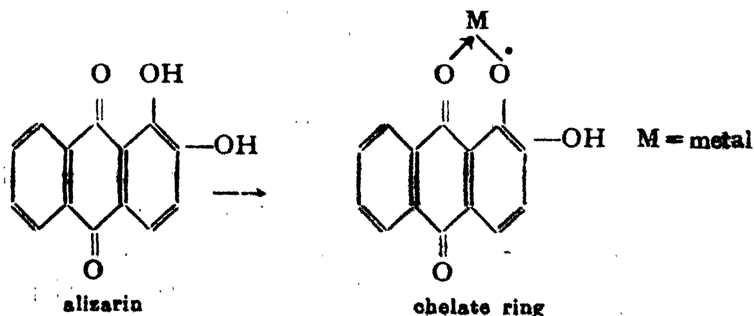
This view of the relation between colour and constitution which involves benzenoid and quinoid structures and the phenomenon of resonance finds further support in the fact that other classes of compounds, in which there is a possibility of a similar resonance also show intense absorption in the visible spectrum and many of them are used as dyes. The best examples of such compounds are the cyanine dyes and phthalo-cyanins.

A further advantage of this view is that it gives a rational explanation of the functions of the auxochromic groups. According to this view, it is clear that the introduction of such groups means the creation of a new state which can participate in the resonating system.

Anthraquinone Dyes

The dyes derived from anthraquinone can be conveniently grouped into three divisions: (*a*) mordant dyes, (*b*) vat dyes and (*c*) acid dyes.

(*a*) MORDANT DYES :—The hydroxy and amino derivatives of anthraquinone constitute this class of dyestuffs. In all these compounds, at least one hydroxyl group is in α-position to the *CO* group. The dyes, thus, combine with metallic hydroxides to form coloured compounds called '*lakes*'. They are formed by the interaction between the dye and the mordant and are unusually stable towards hydrolysis. It is now believed that *chelation* plays an important part in their formation thus :—



alizarin chelate ring

(The presence of the six-membered chelate ring contributes greatly to the stability of the lakes formed).

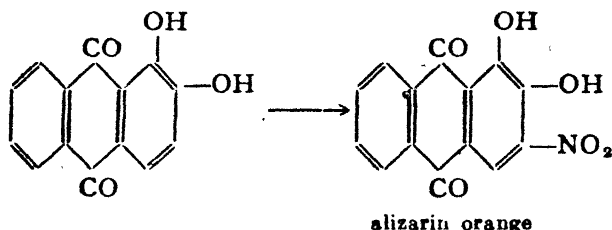
The colour of the lake varies with the nature of the metal. Thus, alizarin, a typical member of this class, gives :—

with $Al(OH)_3$, a *red* lake

with $Cr(OH)_3$, *claret* red and *maroon* lake

with $Fe(OH)_3$ a *violet* lake.

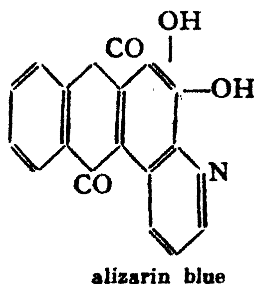
The most important dye belonging to this class is alizarin ; it is a natural dye of the madder root. Another mordant dye of this group is alizarin orange. It is obtained by nitrating alizarin with HNO_3 in presence of H_2SO_4 and boric acid.



alizarin orange

(nitration of dibenzoyl alizarin yields the 4 nitro derivative).

ALIZARIN BLUE :—It is obtained by heating 3-amino-alizarin with glycerol and H_2SO_4.



alizarin blue

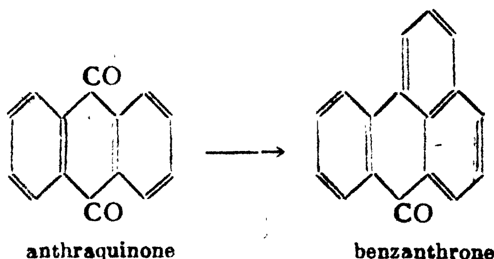
It is a substitute for indigo-blue and is used in the form of its soluble sodium bisulphite addition compound.

(*b*) VAT DYES :—The dyes belonging to this class are at present technically most important. They are very fast and possess brilliant shades. They are applied to the fabric in the same way as

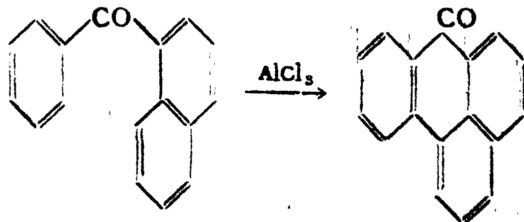
indigoids, from strongly alkalin vats (hence, the name). They are further subdivided into ;—(a) benzanthrone dyes, (b) indanthrene dyes and (c) acyl-amino-anthraquinone dyes.

(a) Benzanthrone dyes, which do not contain *nitrogen* at all, are insoluble in water, but they all contain the *CO* grouping which, on alkaline reduction, passes into *C—OH* group soluble in alkali. They, thus, give the *leuco* compounds in the reducing vat. The typical members are : benzanthrone, violanthrone.

Benzanthrone :—It is prepared by heating anthraquinone with glycerol and concentrated H_2SO_4 in presence of Cu powder.

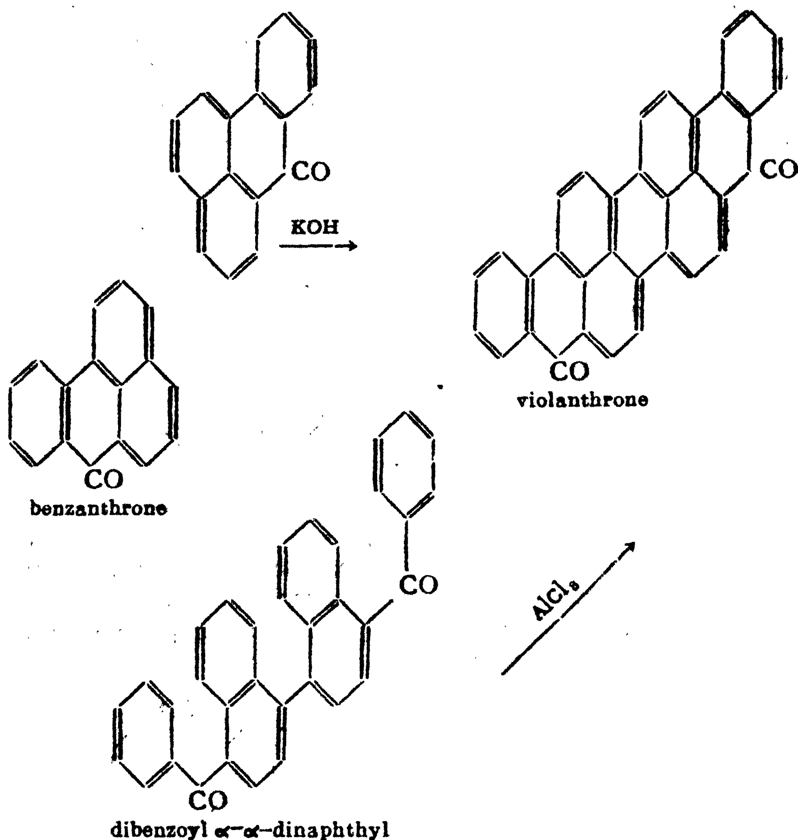


Benzanthrone is also obtained by the *Schol's* condensation reaction ; it consists in heating α-benzyol-naphthalene with anhydrous $AlCl_3$ at 150°C for two hours ; condensation takes place with the splitting of nuclear *H* atoms :—



It is a general reaction ; aromatic ketones (with suitable structure) undergo intramolecular dehydrogenation under the influence of $AlCl_3$. It has been of great synthetic value in the preparation of polynuclear compounds. Beznanthrone constitutes the starting point for the preparation of other dyes of this class *e. g.* violanthrone and caledon jade green.

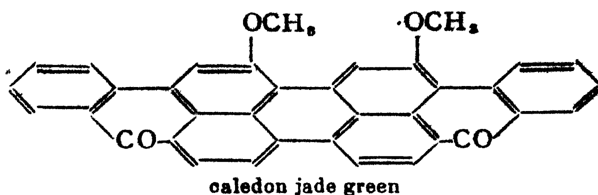
Violanthrone (indanthrene dark blue):—It is obtained from benzanthrone by fusion with *KOH* or by the action of $AlCl_3$ on dibenzoyl α-α-dinaphthyl:



(The latter synthesis establishes the constitution of the dye).

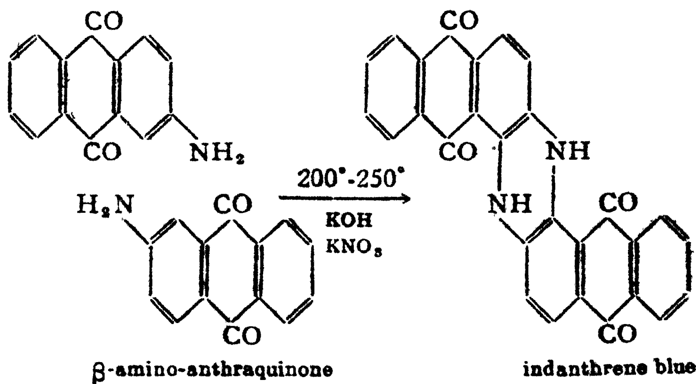
Violanthrone produces a deep violet shade which is extremely fast to light, water and bleaching.

Caledon jade green is an important modern dye. It is produced by the oxidation of dibenzanthrone with MnO_2 and H_2SO_4 to its dihydroxy-derivative which is then methylated with $(CH_3)_2SO_4$.



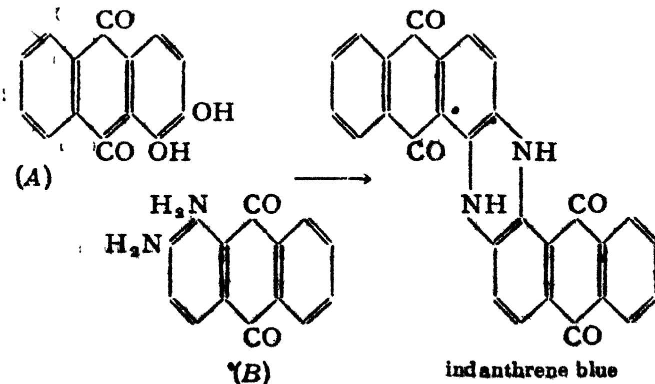
caledon jade green

(b) *Indanthrene dyes*:—These contain *N* as part of a ring system. *Indanthrene* is the most important dye of this class. It was obtained by R. Bohn by fusing β-amino-anthraquinone with alkali:—



β-amino-anthraquinone indanthrene blue

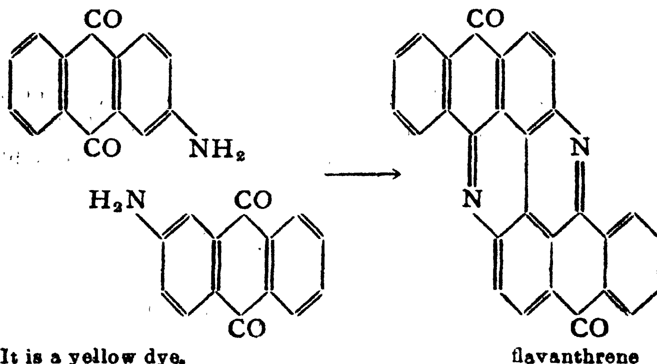
It is also obtained by heating alizarin (*A*) with 1-2-di amino-anthraquinone (*B*):—



indanthrene blue

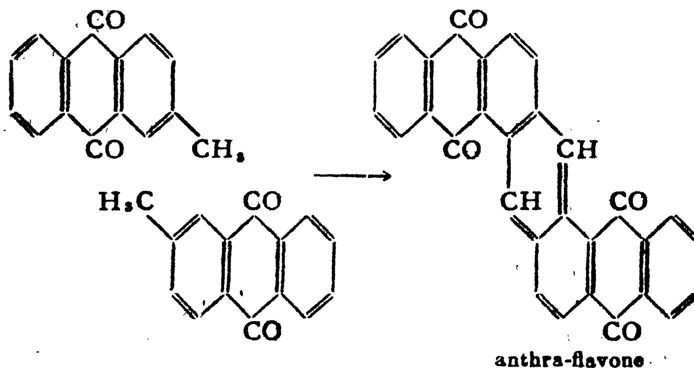
It is very fast to light and washing.

Flavanthrene:—It is formed along with indanthrene blue, when β-amino-anthraquinone is fused with alkali, especially at higher temperature above 270°.



It is a yellow dye. flavanthrene

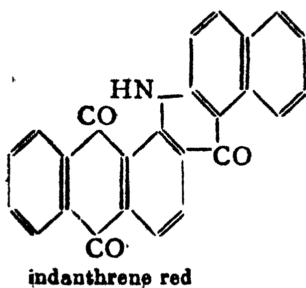
A dye which closely resembles the indanthrene dyes in structure is *anthra-flavone G* (greenish yellow). It is obtained by heating β-methyl-anthraquinone with alcoholic KOH at 150°. Partial oxidation and condensation take place simultaneously:—



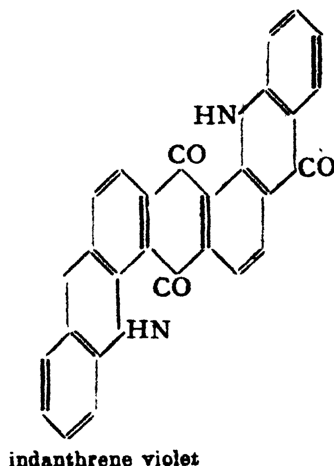
anthra-flavone

(c) Acylamino anthraquinone dyes or algol dyes. They are aroylamino anthraquinones; they were introduced by Bohn as vat dyes. The representatives of the class are algol yellow which is 1-benzoyl-amino-anthraquinone; indanthrene yellow GK is 1·5 dibenzoylamino-anthraquinone and algol red is the dibenzoyl derivative of 1·4 diamino anthraquinone.

(*d*) ANTHRAQUINONE—ACRIDONE DYES :—They contain an acridine system fused to anthracene system. Two important dyes of this class have been prepared. They are indanthrene red and indanthrene violet.

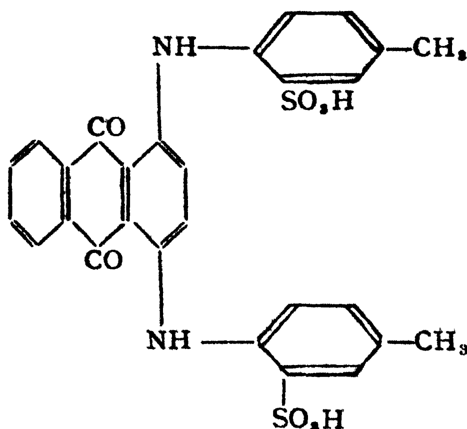


indanthrene red



indanthrene violet

(C) ACID DYES :—They are the sulphonic acid derivatives of amino anthraquinones. They are obtained by heating the hydroxy-derivatives of anthraquinone with ammonia or substituted ammonias (NH_2R) under pressure. They contain an NH_2 group in α-position to CO group. A typical dye of this class is *alizarin cyanine green* :—

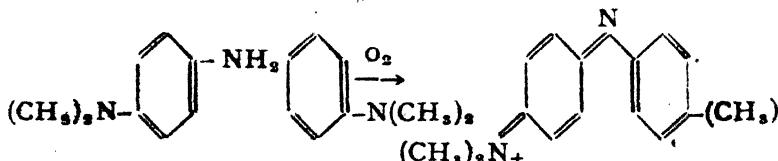


It is obtained by heating quinizarin or 1·4 dichloroanthraquinone with *p*-toluidine with or without boric acid and subsequent sulphonation to render the dye soluble. Sulphonation takes place on the *p*-toluidine nucleus.

Quinone-Imine Dyes

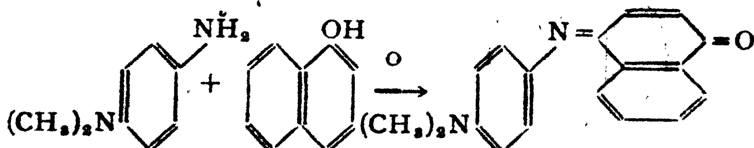
The dyes belonging to this class may be subdivided into: (*a*) those derived from *p*-quinone-imine and (*b*) those derived from *o*-quinone-imine. The ***indamines*** and ***indo-phenols*** represent the first sub-division, while the *oxazines*, ***thiazines*** and ***azines*** constitute the second.

(A) *Indamines* :—They are formed by oxidation of an equimolar mixture of a *p*-diamine with a free NH_2 group and monoamine with a free *para* position. Bindschedler's green is, thus, obtained from dimethyl-*p*-phenylene-diamine and dimethylaniline by oxidation with $K_2Cr_2O_7$ and acetic acid :—



They form *blue* or *green* salts ; but are very sensitive to acids. Hence, they are not used as dystuffs, but serve as starting-materials for the thiazine, azine and sulphur dyes.

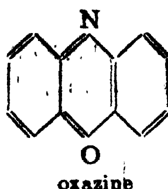
Indo-phenols :—They are obtained when a *p*-diamine with a free NH_2 group is oxidised in presence of a phenol with a free para position. The typical member is *indophenol,* obtained by oxidation of a mixture of α-naphthol and dimethyl-phenylene-diamine:—



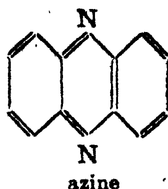
They are used for the preparation of sulphur dyes.

B. The dyes derived from *o*-quinone-imine are very comprehensive and technically important. They contain a heterosystem

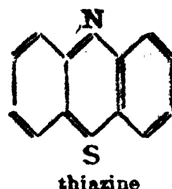
with O, N or S as part of a condensed system. The following are the parent compounds from which the dyes of this class are derived—



oxazine



azine



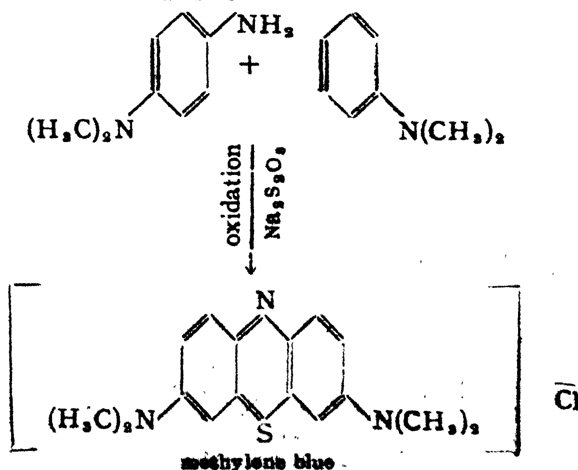
thiazine

The dyestuffs are obtained by introducing auxochromes in *para* position to the N atom. The common auxochromes so introduced are NH_2 NR_2. OH. The chromophore of the dyes is the *o*-quinoid grouping.

THIAZINES :—They are related to diphenylamine; thiazine, the parent compound, is obtained from fusing diphenylamine and sulphur. An important member of this class is the dye, ***Lauth's violet*** which is 3–9-diamino-phenazine. It is obtained from *p*-phenylene-diamine and hydrogen sulphide by oxidation with $FeCl_3$.

Methylene blue is the tetra-methyl derivative of Lauth's violet. It is prepared by oxidising dimethyl-*p*-phenylene-diamine with $FeCl_3$ in presence of H_2S.

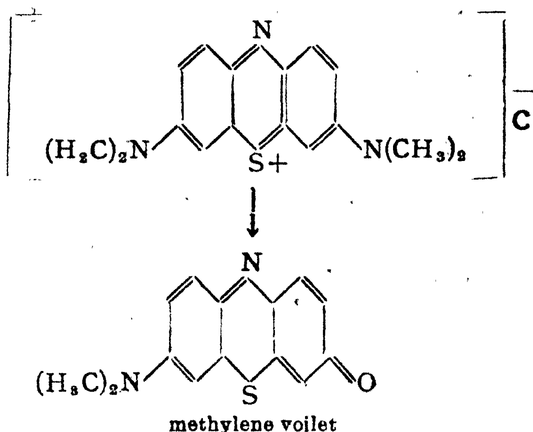
It is also obtained according to Brenthsen by oxidation of equimolar quantities of dimethyl-*p*-phenylene-diamine and dimethylaniline in presence of $Na_2S_2O_3$:—



methylene blue

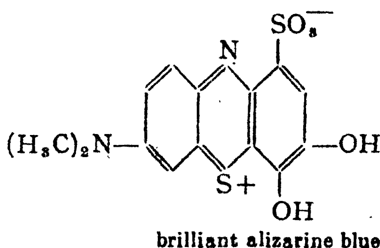
It is used as a dye for tanning-mordanted cotton. It finds application as an indicator and for staining purposes. On account of the last property it is called 'vital' dye.

A number of dyes have been obtained from methylene blue by the help of simple reactions. Thus, alkaline hydrolysis of the dye yields the indophenolic dye, methylene violet.



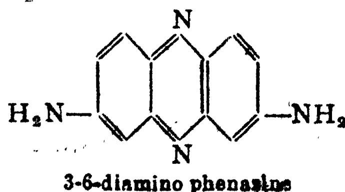
methylene voilet

Brillant alizarin blue is an important dye of this class:—



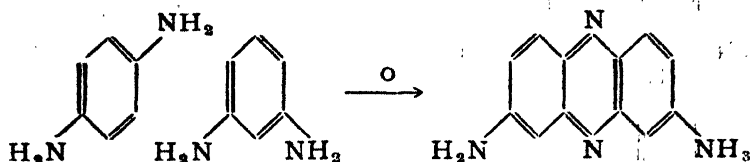
brilliant alizarine blue

AZINES OR PHENAZINES :—They are the nitrogen analogues of thiazine : the simplest member of this class is :—

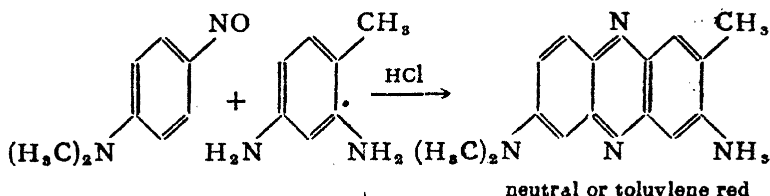


3-6-diamino phenazine

They are usually obtained by oxidation of a mixture of a *p*-diamine and an *m*-diamine in acid medium :—

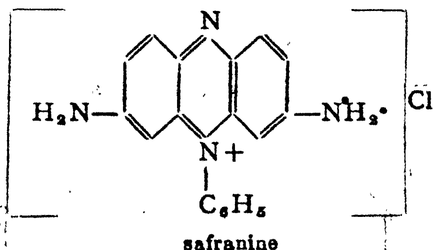


or nitroso-dimethyl aniline is condensed with *m*-diamine in acid solution, *neutral red* is thus obtained :—



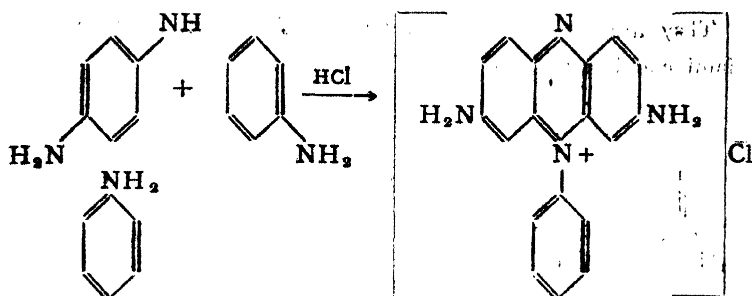
neutral or toluylene red

SAFRANINES :—They are the most prized dyes. They are very fast to light and washing. They dye tannin-mordanted fabrics from red to violet shades. Structurally, they are the diamino-derivatives of phenyl-phenazonium salts. Safranine, the most simple and typical of them has the structure :—



safranine

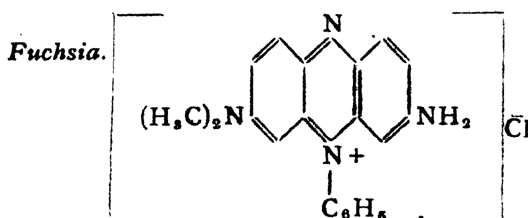
It is obtained by oxidising *one* molecule of *p*-phenylene-diamine with *two* molecules of a mono-amine in acid solution :—



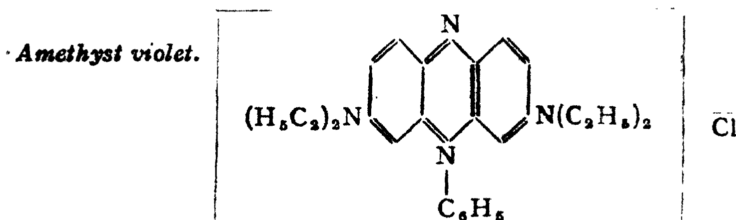
(The diamine must contain a free NH_2 group and one of the mono-amines must be a primary amine).

Some important safranines are :—

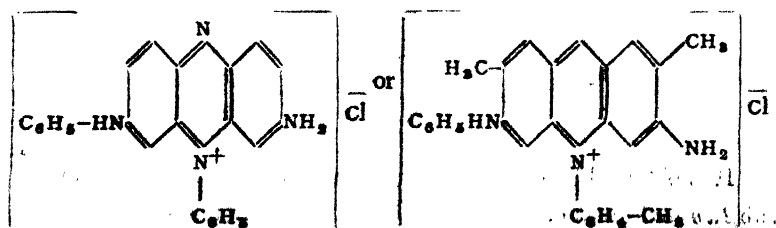
Fuchsia.



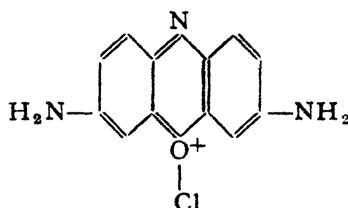
Amethyst violet.



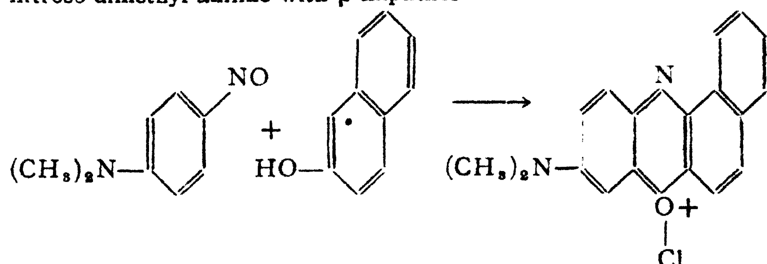
Mauveine:—(Perkins' mauve), the *first* synthetic dye, is a safranine:—



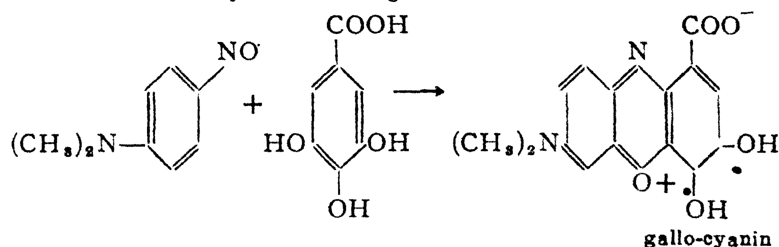
Oxazines:—These are the oxygen analogous. The simplest dye of this class is:—



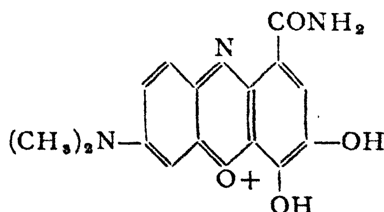
These dyes are obtained by condensation of nitroso-dimethyl aniline or nitroso-phenols with alkylated *m*-amino-phenols or naphthols. Naphthol blue or Meldola blue prepared by condensing nitroso-dimethyl-aniline with β-naphthol:



Resorcinol, pyragallol and gallic acid are employed in the preparation of important oxazines. Thus gallocyanin is obtained from nitroso-dimethyl-aniline and gallic acid:—

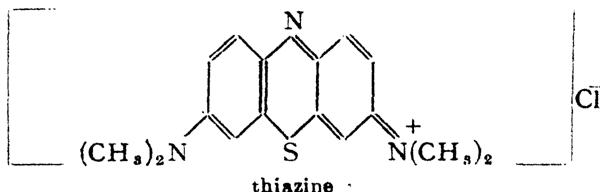


Gallamine blue:—It is the amide derivative of gallocyanin:—



The phenocyanin dyes are also obtained from gallocyanin and phenols by heating. They are much used in calico printing.

STRUCTURE OF THE THIAZINES, OXAZINES AND AZINES :— In the foregoing pages, these dyes have been structurally represented as derived from *ortho*-quinone amine. A *para*-quinoid structure is also possible but so far it has not been possible to determine with certainty the exact structure of these dyes. The *para*-quinoid structure for the thiazine derivative would be :—



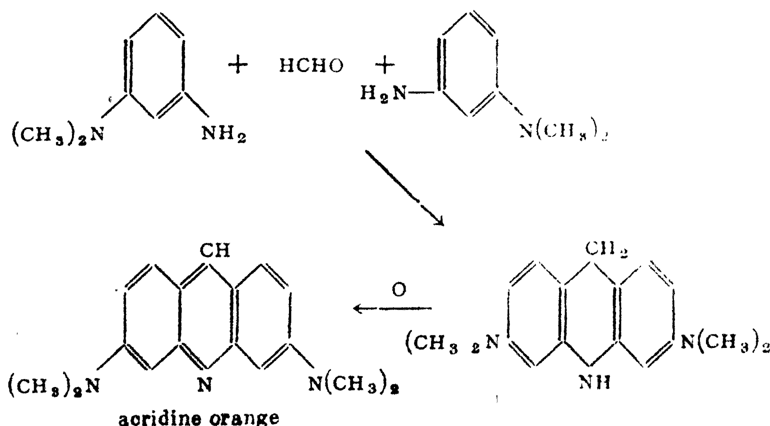
thiazine

Similarly, para-quinoid structures for oxazines and azines may be formulated.

Acridine Dyestuffs

They are derived from acridine. Two important dyes are acridine orange and trypaflavine.

ACRIDINE ORANGE :—It is obtained by heating together dimethyl-*m*-phenylene-diamine and formaldehyde and subsequent oxidation :—

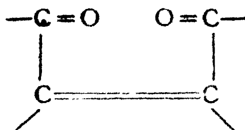


acridine orange

Trypa-flavine is the metho-chloride of 2–8-diamino-acridine *i. e.* proflavine. It is also known as acriflavine and finds extensive use as a powerful antiseptic in modern medicine. It is not toxic and does not retard the process of healing.

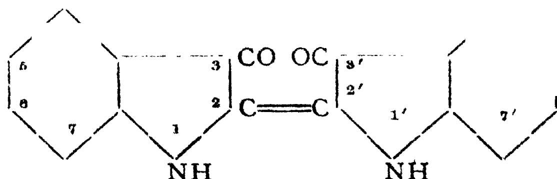
Indigoids

Indigo contains as its chromophore the grouping :—



which is the cause of its colour. Friedlander has proposed that dyes which possess this grouping be called "indigoids." Hence they include the halogenated indigo derivatives and certain related compounds of analogous structure *e. g.* thio-indigo and its derivatives. They are the results of the several attempts made to modify the shade of the natural dye, by introducing useful variants in the structure of the molecule. These variants include introduction of : (*i*) halogens in the molecule giving rise to halogenated indigos; (*ii*) of naphthyl residues in place of benzene nuclei, and (*iii*) of replacing NH by S or O, and lastly (*iv*) the replacement of an indoxyl nucleus by a thio-indoxyl one or any other suitable nucleus of acenaphthaquinone.

HALOGENATED INDIGOS :—Chloro and bromo-derivatives are known ; they are characterised by their great fastness and brilliancy of shades. The shade appears to depend on the number and position of the halogen atoms in the molecules : the nomenclature adopted for the derivatives is :—

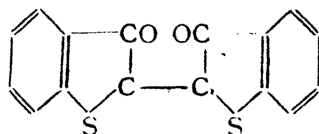


The following are some of the typical commercial products :— *Ciba blue B* is 5-5′-7-tri-bromo-indigo ; *Ciba blue 2B* is 5-5′-7-7′

tetra-bromo indigo; the *Tyrian purple*, or *Royal purple*, the imperial dye of the Romans, is the 6-6′-di-bromo-indigo. At present, this particular dye is not manufactured but the abovementioned *Ciba blue 2 B*, obtained by the direct bromination of indigo constitutes an important vat dye.

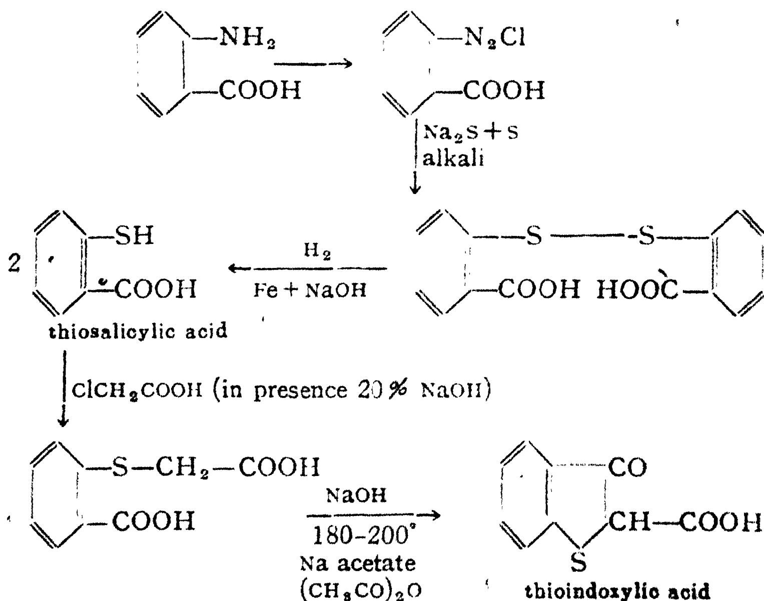
NAPHTHALENE INDIGOS :—Replacement of the benzene nuclei by naphthalene residues gives rise to dyes of green shade but they are not fast and hence have found no technical applications.

Similarly, the oxy-indigos obtained by replacing the NH-group by—O—are yellow compounds with little or no tinctorial properties at all. On the other hand, the replacement of NH—by—S—gives rise to dyes of much brighter shades. They are called *thioindigos*. They include halogeno-bromo and ethoxy-derivatives of the parent compounds.

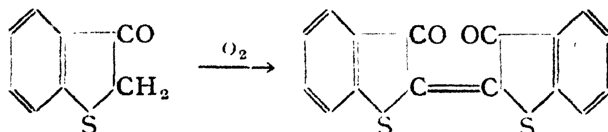


thioindigo

This indigo has been obtained as follows, starting from anthranilic acid :



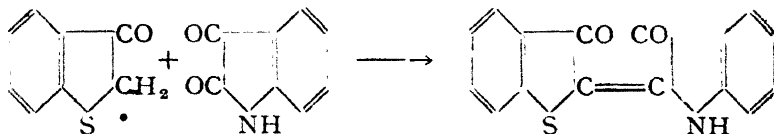
The latter is decarboxylated to thioindoxyl—which on aerial oxidation, is converted into thio-indigo.



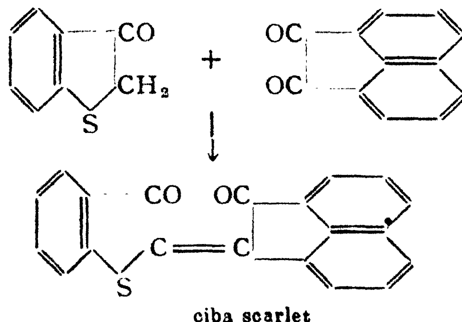
Thio-indigo is faster than indigo, and possesses a bluish red shade. The 5-5′-di-bromo- and 6-6′-diethoxy derivatives give brighter and faster shades. Thio-indigo and its derivatives are applied to the fabric in the same way as indigo.

Many allied vat dyes are now made available which contain two different types of nuclei. One of the units is usually the thio-indole unit: two typical of such dyes are thio-indigo scarlet R and Ciba Scarlet.

Thio-indigo Scarlet R is obtained by fusing together thio-indoxyl and isatin :



Thio-indoxyl and ace-naphthaquinone give *Ciba scarlet.*

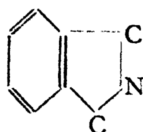


ciba scarlet

This dye is remarkably fast and possesses a very brilliant shade.

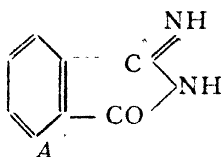
PHTHALOCYANINS :—We have so far described in some details the most important blue and red or scarlet dyestuffs which are derivatives of indole and thio-indole system. Recently, Linstead and

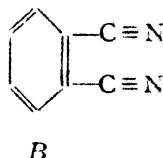
Low have discovered new type of blue to green dyestuffs. They have been called '*phthalocyanins*' and contain an isoindole nucleus :-



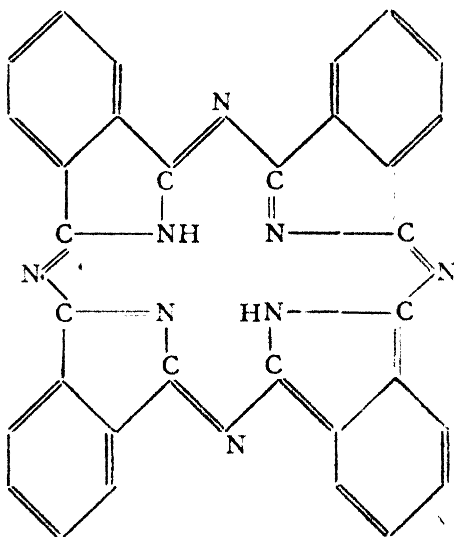
iso-indole nucleus

They are obtained by the action of metallic compounds like Cu_2Cl_2, CuO, MgO or metals like Cu or Mg on imido-phthalimide (A) or phthalo-nitrile (B).





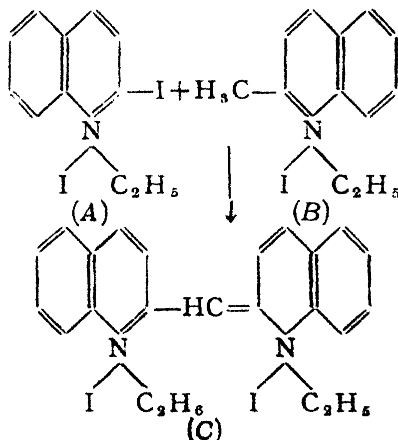
They are highly complex compounds in which the metallic atom is probably present as a complex. The metal-free compound has been assigned the following formula ; it is based on X-ray data. In the 16-membered ring, the atoms show a spacing which indicates a resonating structure.



Thus, because of the resemblance of the above skeleton with that of *porphyrins*, the phthalocyanins are also termed *porphyrazines*. They are characterised by great stability towards acids, bases and even heat. They hence find application as pigments in many fields. The copper compound is known as monastral blue, gives a very pure blue useful in printing inks, paints and lacquers. Halogenated and sulphonated phthalocyanins are also known. The halogenated derivatives give green dyes; the sulphonated phthalocyanins being soluble in water are capable of being used as typical dyes.

Cyanine Dyes

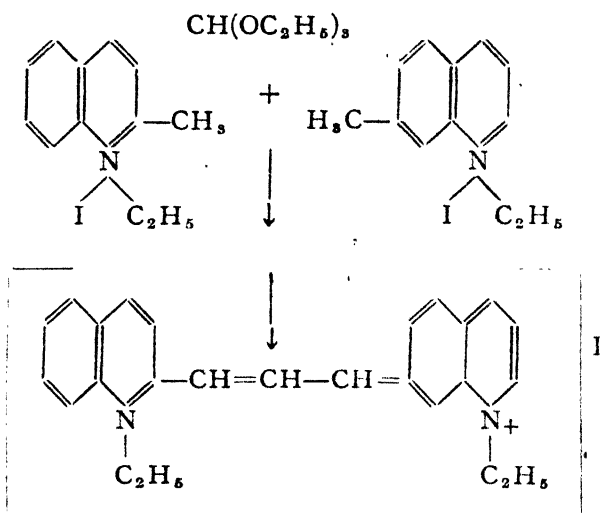
When a mixture of 2-iodo-quinoline ethiodide (*A*) and quinoline ethiodide (*B*) is treated with alcoholic potash, the 1-1-diethyl *p*-cyanine iodide (*C*) is formed:



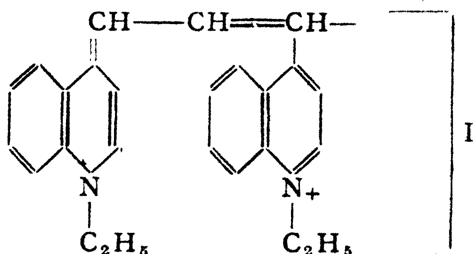
The above compound and similar ones which contain two heterocyclic nuclei, each with a nitrogen atom, and separated by an odd number of carbon atoms, which form a conjugated system, are substances which dye a blue colour. However, they cannot be used as dyestuffs as they are extremely fugitive. But they are of great value as *sensitisers* in photo-chemistry and in colour photography because of their great sensitiveness to light. The sensitivity of the

dye depends on the length of the carbon chain separating the two hetero-nuclei; the *longer the conjugated chain*, the *greater* is the *sensitisation* towards the *red end of the spectrum.*

One of the typical cyanin dye is *pinacyanol*, sensitol red. It is obtained by the condensation of quinaldine-ethiodide and ethyl formate in presence of pyridine :—



Another important member of this group is cryptocyanine:



The cyanine dyes are too costly to be used as dyes in the textiles, they find application in photography. Cryptocyanine is used to take pictures with infra red radiant energy. It is thus specially valuable for aerial photography.

Sulphur Dyes

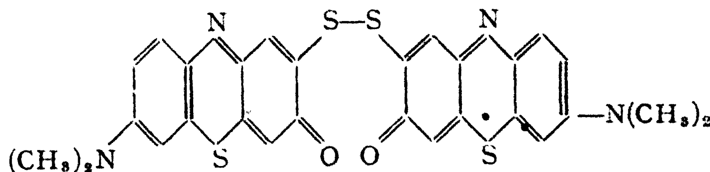
Sulphur dyes constitute some of the most important technical dyes. They are remarkably fast and are cheap. Hence, they find extensive application. Structurally, they are very complex; but they have been roughly divided into two classes :—

(a) The blue and black dyes probably with a *thiazine* structure.

(b) The yellow to brown dyes derived probably from the *thiazole* system.

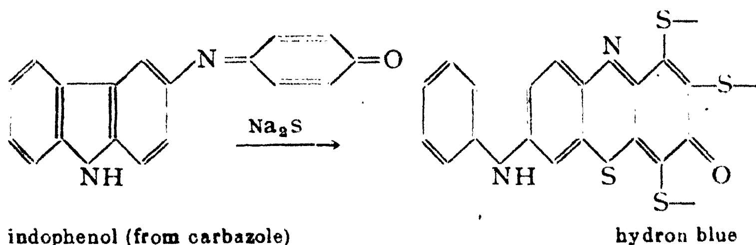
They are all obtained by fusing various organic compounds with sulphur, or alkali poly-sulphides (Na_2Sx). The first sulphur dye was obtained by Croissant in 1873 by fusing organic waste matter with alkali sulphide. Subsequently, Vidal (1893) obtained the technically important dye, Vidal Black, by fusing *p*-amino phenol with sodium sulphide. This has been followed by a number of sulphur dyes which are blue, green, yellow or brown. They are applied to the fabric from an alkaline reducing vat. Usually, Na_2Sx is employed to convert the insoluble dye into the soluble reduction product in the vat.

IMMEDIAL BLUE :—Obtained from methylene violet, it has been assigned (tentatively) the following structure :—



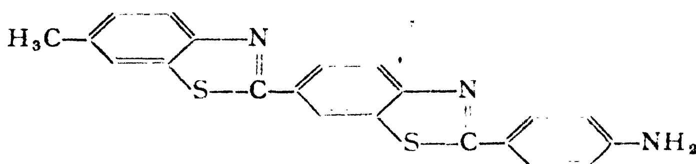
It is highly probable that all such dyes possess the di-sulphide —S—S—grouping or the mercapto group *SH*—linked to a thiazine system. In confirmation of this view, it has been found that indo-phenols and indamines have proved convenient starting-points for the preparation of the blue and black sulphur dyes.

Hydron blue :—It is the most important blue sulphur dye. It is obtained by heating the indophenol with sodium sulphide :—



indophenol (from carbazole) hydron blue

The yellow dye which is best known is *primuline yellow*. It is prepared by heating *p*-toluidine with sulphur 220°. Probably a mixture of compounds with thiazole rings like,



is formed. It is again treated with *sulphur* to render it soluble. Usually it is diazotised on the fabric and coupled with β-naphthol when a beautiful red shade is obtained. The latter is very fast.

Sulphur black :—This is the most largely used of the sulphur dyes. It is obtained by boiling together 2, 4-dinitrophenol with an aqueous solution of *Na*-polysulphide. It is dyed from an alkali sulphide bath.

CHAPTER XII

SYNTHETIC MACROMOLECULES

Introduction :—Nature has given us a limited number of compounds like cellulose, rubber and proteins which are composed of giant molecules; they are called macromolecules by Staudinger. They possess very valuable properties like great mechanical strength, elasticity and a resistance to wear and tear. Hence they find extensive use in making clothing, tyres, films, protective coatings etc. But such natural materials are limited in number and also possess some inconvenient properties of insolubility in water and other organic solvents. Recently therefore, much effort has been expended to overcome both these limitations. The problem has been tackled from two different stand-points; (1) The modification of the natural macromolecules by esterification, etherification etc. to obtain derivatives with marked change in physical properties, especially in solubility, and (2) the syntheses of new types of macromolecules modelled on the natural ones, and starting from cheap raw materials; this has actually led to the development of the modern mammoth industries—the plastics industry, the synthetic fibre industry etc.

Classification.—In this way, man has been able not only to add to the list of the few natural macromolecules but also has produced molecules with highly desirable properties and of great utility. These can be classified under four big divisions :

(*a*) Synthetic Rubbers,
(*b*) Synthetic Fibres,
(*c*) Synthetic Plastics and Resins,
(*d*) Silicones.

The division is arbitrary and is based on the general chemical composition and on the uses to which the synthetics are put.

Synthetic Rubbers

An early discovery made by Tilden in England, and by Harries in Germany, was that isoprene, a product of pyrolysis of natural rubber or of turpentine, could polymerise to a rubber-like product. The polymerisation was observed to take place on standing alone or

in the presence of metallic Na. During the World War ,I, the German attempted to obtain synthetic rubber by this method, to meet the requirements for the natural product. However, there was no cheap method available for the quantity production of isoprene, and hence the above reaction could not be commercialised. However, Kondakoff had sometime, noticed that the conversion of 2, 3 dimethyl butadiene to a rubber like product, took place when heated with KOH. The Germans developed a cheap method for the commercial production of this compound from acetone. It was then used to produce the synthetic rubber substitute called 'methyl rubber'. Two methyl rubbers H and W were made available by using two methods of polymerisation. However, the methyl rubbers were found to be poor substitutes. A methyl rubber tyre did not last for more than 200 miles and the inner tubes wore out even after a few hundred miles. This was the beginning of synthetic rubber industry which now constitutes an important industry in U. S. A.

Classification.—At present, the synthetic products include a large variety of polymers called *elastomers*. They are capable of being stretched at least 150 per cent of their original length without breaking and then return to approximately their original shape unaided. An arbitrary classification is based on the general chemical composition of the product. Thus we have: (1) Elastoprenes and (2) Elastoplastics.

(*a*) The elastoprenes are derived from butadiene, its homologues and analogues. Structurally, they are *linear* polymers formed from the *dienes* through 1, 4 polymerisation; further they have been subdivided into (1) Simple polymers—a single monomer is subjected, to polymerisation and (2) Co-polymers—when a mixture of two or more different monomers is polymerised. They are not mechanical mixtures but both the component molecules are incorporated into the chains which comprise the macromolecule. The unsaturated compounds commonly used for the production of co-polymers are styrene, acrylonitrile etc.

(*b*) Elastoplastics—they include (1) polyvinyl derivatives (2) polyacrylic esters and (3) thiokohls.

The actual production of synthetic rubbers involves three important steps. They are: (1) the quantity production of *dienes*

and mostly from cheap, readily available, and almost inexhaustible raw materials, (2) the polymerisation of the diene; this may comprise a simple polymerisation of a simple diene like butadiene or other diene with different mono-enes, (3) vulcanisation of the product *i,e.* the polymer to improve its properties, especially its ability to return to its original shape.

The most important dienes and monomers so far used technically in the synthetic rubber production are—

(1) 1, 3 Butadiene, isoprene and 2, 3 Dimethyl butadiene.

(2) Chloroprene, Vinyl chloride, Vinyl acetate and butenes.

(3) Styrene, acrylonirtile (used in the preparation of copolymers only).

DIENES AND MONOMERS :—The sources and the methods of preparing the dienes on a large-scale shall be discussed in some detail.

Butadiene.—The sources for the production of butadiene are many and varied. Thus butadiene is now obtained from (1) fermentation of grain, (2) acetylene, (3) coal-tar and (4) petroleum.

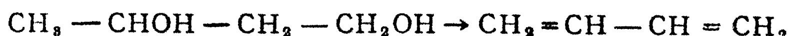
(1) The fermentation of grain or starchy material gives (*a*) ethyl alcohol, (*b*) butyl alcohol or (*c*) 2, 3 butylene glycol depending on the use of specific ferments and other experimental conditions. The production of butadiene from these alcohols involves a series of reactions.

(*a*) In the Russian method, ethyl alcohol (in the form of vapour) is passed over a mixed catalyst consisting of Al_2O_3—ZnO.



$$2C_2H_5OH \longrightarrow CH_2 = CH - CH = CH_2$$

Probably, acetaldehyde is first formed which reacts with an alcohol molecule which is dehydrated to give butadiene :

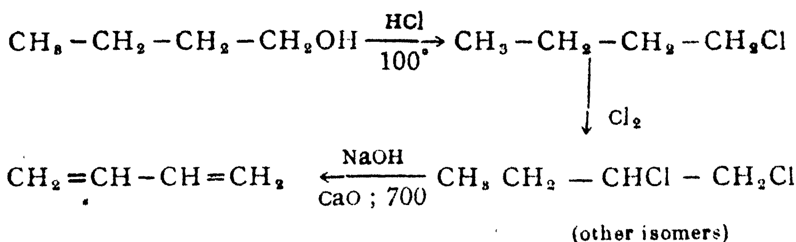


$$CH_3 - CHOH - CH_2 - CH_2OH \rightarrow CH_2 = CH - CH = CH_2$$

In the recent American process, which is a variant of the above, a mixture of acetaldehyde and alcohol is passed over a mixed catalyst. The yield is about 70 per cent.

(*b*) An English method uses butyl alcohol obtained from the fermentation of starch by the Fernbach bacteria, as the source of

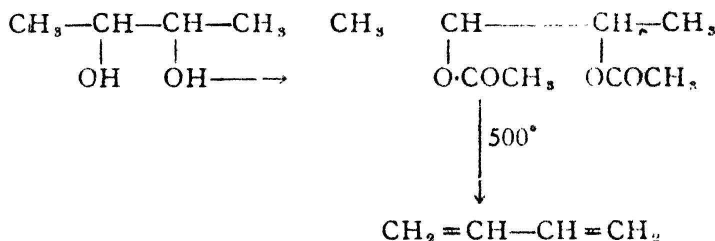
butadiene. The conversion is effected according to the following scheme :



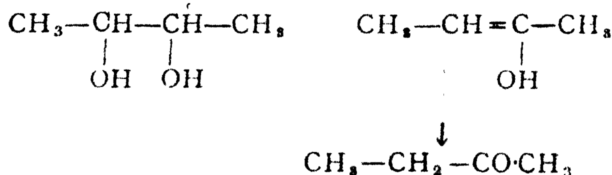
Butyl alcohol is also obtained by a catalytic method which involves the passing the vapours of alcohol over BaO at 400 to 500°.

$$CH_3-CH_2OH+CH_3-CH_2OH \longrightarrow CH_3-CH_2-CH_2-CH_2OH$$

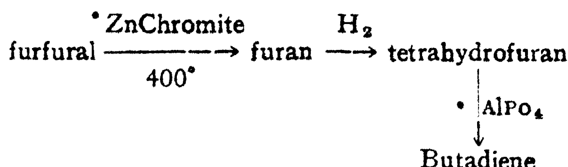
(*c*) A new promising method makes use of 2, 3 butylene glycol —a product of grain fermentation with—Acrobactor acrogenes or Acro Bacillus polymysea as a raw material for butadiene. The glycol is first acetylated with acetic anhydride and the di-acetyl derivative is deacetylated catalytically to give butadiene.



Direct dehydration of the glycol is unsatisfactory, because of the formation of a large amount of methyl—ethyl ketone.

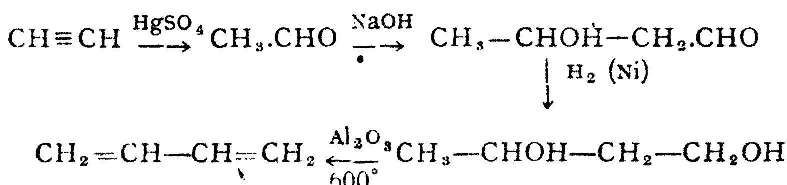


In another method, butadiene is obtained from the corncobs : the corncobs are distilled with dilute H_2SO_4 to give furfural ; the latter is changed by a series of reactions into butadiene.



(2). Acetylene continues to be the important single source for the quantity production of butadiene. It is Germany, that has developed a number of methods of preparing butadiene, starting from acetylene. The chemistry of the different methods is described here.

(*a*) In the earliest method, acetylene was first converted into 1, 3 butylene glycol which was dehydrated to butadiene.

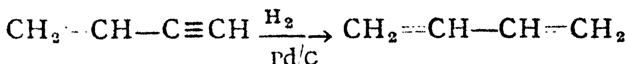


The yield in the last step is found to be quantitative.

(*b*) In the second method, acetylene is first dimerised in presence of ammoniacal Cu_2Cl_2, to vinyl acetylene.



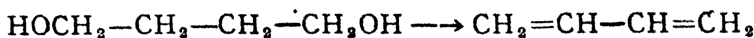
The latter on partial catalytic hydrogenation gives butadiene.



(*c*) The third method was developed recently by Reppe in Germany. It is a part of the modern acetylene chemistry which Reppe has been able to found and develop. In this method, formaldehyde is condensed with acetylene to form butine—2 diol 1, 4, in presence of cu-acetylide.

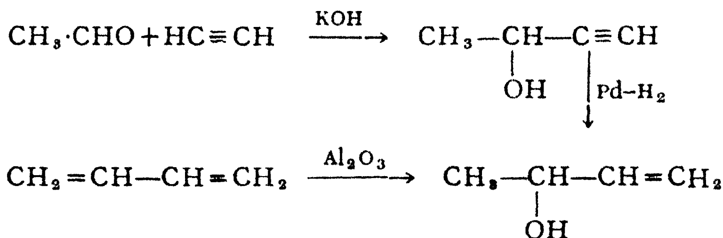
$$CH_2O + HC{\equiv}CH + CH_2O \xrightarrow{90\text{-}100^\circ} HOCH_2{-}C{\equiv}C{\cdot}CH_2OH$$

which is catalytically reduced to 1, 4 butylene—glycol and subsequently dehydrated to butadiene.

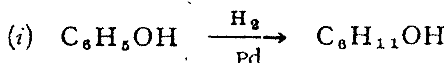


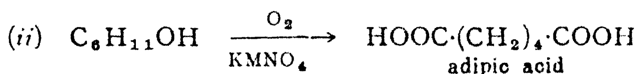
The yields are quite satisfactory.

(*d*) In the fourth method, the principle of Reppe addition of acetylene to aldehydes in presence of solid KOH is utilised. The steps involved are:



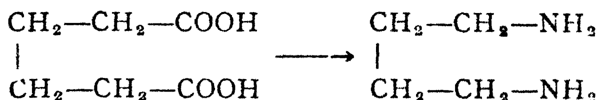
(3) The coal-tar—the source of the numerous aromatics—dyes, drugs, solvents, flavouring agents etc., has been sought for as a source for butadiene. The constituent of coal-tar which is used in the production of butadiene is phenol. The chemistry involved in the several steps is outlined as follows :—





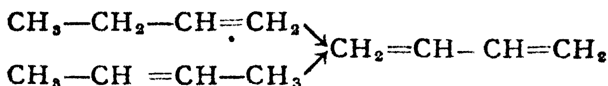
Adipic acid is also obtained from tetrahydrofuran. The latter is treated with HBr to give the 1·4 dibromobutane which on reaction with KCN and subsequent hydrolysis forms adipic acid.

(*iii*) The adipic acid is esterified, amidated and the diamide subjected to Hoffman degradation to give the diamine.

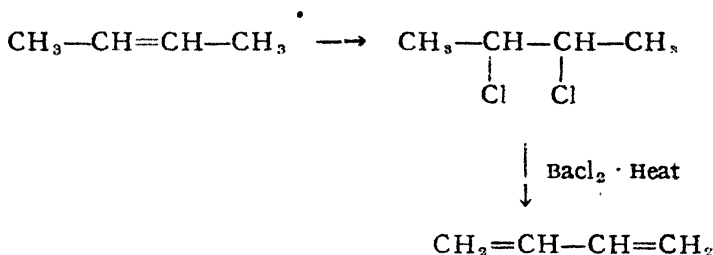


(*iv*) The diamine is methylated and then the quaternary iodide dry distilled in presence of Ag_2O when the di-olefin, butadiene is formed.

(4) Petroleum forms the sole raw material for the large-scale production of butadiene, in U. S. A. The cracking of petroleum produces a small quantity of butadiene, which is extracted with furfual a specific solvent for butadiene Much of it however, is obtained from butene (1 and 2), by catalytic dehydrogenation; the catalysts used are Al_2O_3 and Cr_2O_3 and the temperature range is 600 to 650 under reduced pressures.



In another process, the butenes are first converted into their dichlorides or dibromides which are then dehydrohalogenated under catalytic conditions to give butadiene.



Butadiene is a gas (b.p. 1). It gives the usual 1·4 addition reactions of a di-olefine.

Isoprene.—The first synthetic rubber was the rubbery material obtained by the polymerisation of isoprene. The latter was first obtained by the pyrolysis of oil of turpentine, which is rather an expensive source, for quantity production. Subsequently, isoprene has been obtained from:—

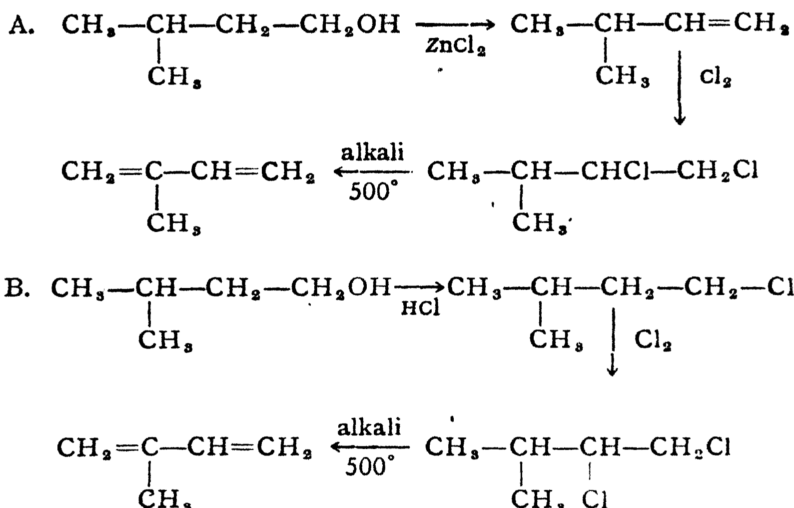
(1) Isoamyl alcohol of the fermentation origin,

(2) acetylene,

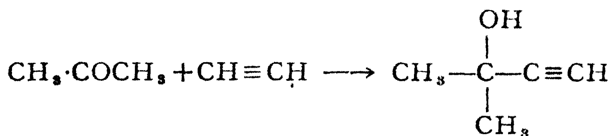
(3) coal-tar,

(4) Petroleum.

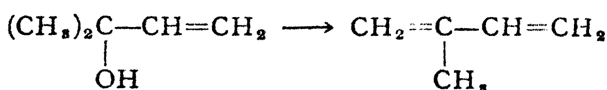
(1) The amyl alcohol from the fermentation of grain can be converted into isoprene in two different ways:—



(2) Acetylene has also been used as a source material for isoprene production. Acetone and acetylene are condensed together in presence of $NaNH_2$ or solid KOH to give a butyne derivative.

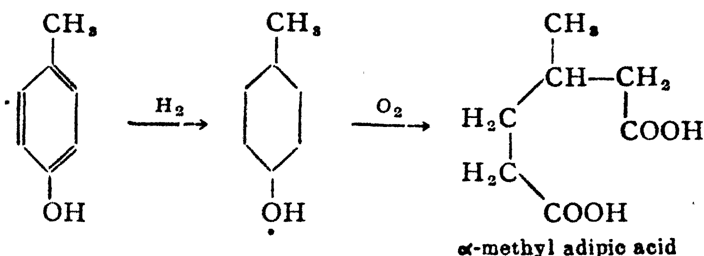


the latter is partially hydrogenated to $(CH_3)_2C(OH)—CH=CH_2$ and subsequently dehydrated to isoprene.



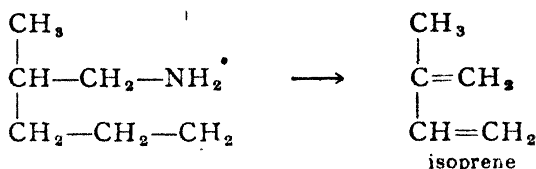
(3) The coal-tar has also been utilised as a source for a raw material that could be readily converted into isoprene. The *m*- and *p*-cresols have been utilised in the production of isoprene. The

methods used are analogous to the one used in the conversion of phenol into butadiene.



α-methyl adipic acid

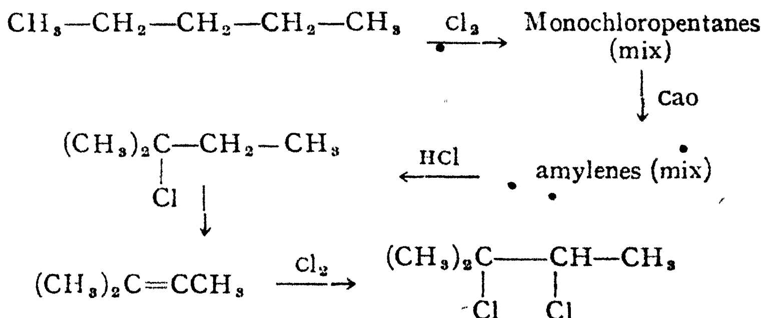
The latter is esterified, amidated and degraded to the corresponding diamine; on exhaustive methylation in the usual way, the di-olefine isoprene is formed:—



isoprene

m-cresol is converted in a similar way into isoprene.

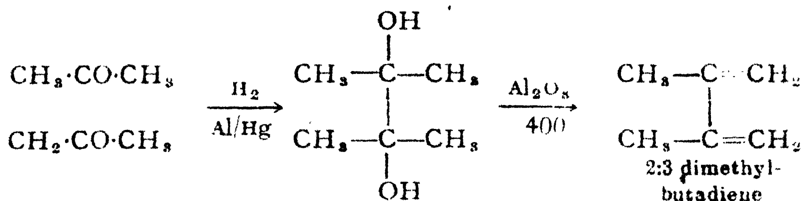
(4) The pentanes from petroleum have been utilised as a raw material for isoprene production. The chemistry involved in the conversion is as follows:—



The latter on dehydro-halogenation with soda-lime at high temperature gives isoprene. It is liquid b. p. 37°. But the process involves many operations which involves considerable losses and hence is not economical. In fact, so far, no cheap method for the quantity

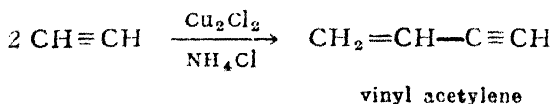
production of isoprene has been successfully developed. There is also another reason which has militated against the production of synthetic rubbers from isoprene. It has been observed that isoprene for rubber production, has to be extremely pure, otherwise, polymers of low molecular weight only, are formed, which have not the requisite physical properties of the natural isoprene rubber.

2·3 Dimethyl-butadiene:—Dimethyl-butadine was utilised as the starting material by the Germans during the first World War to produce synthetic rubber. The dimethyl butadiene can be relatively easily obtained. It is obtained from acetone in the following way: Acetone is reduced by magnesium or aluminium amalgam to pinacol. The reduction is a bimolecular one. The pinacol, which is a dihydric alcohol, on dehydration, gives 2·3 dimethyl butadiene.



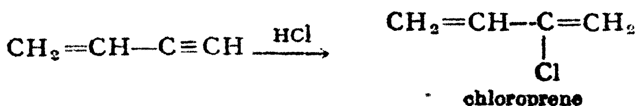
It is a liquid, b.p. 70°. On polymerisation, it forms methyl rubber.

Chloroprene is the monomer used for the production of the synthetic rubber neoprene or duprene—a product developed by the Du Pont Nemours in U.S.A. It is obtained from acetylene as follows :—

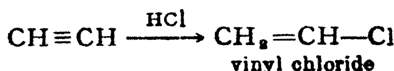


The polymerisation is carefully controlled, otherwise a by-product divinyl acetylene $CH_2=CH—C\equiv C—CH=CH_2$ is formed which finds use as S. D. O i.e. synthetic drying oil.

The vinylacetylene on treatment with HCl gas in presence of Cu_2Cl_2 and NH_4Cl gives chloroprene (the triple bond is preferentially attacked by HCl).

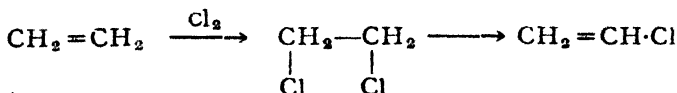


Vinyl chloride is a compound of great reactivity and yields derivatives of far-reaching technical and strategic importance. It can also be conveniently handled and transported. It is obtained from acetylene and hydrogen chloride in presence of catalyst.



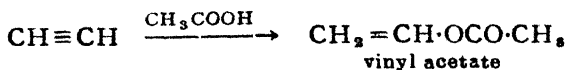
The catalysts commonly used are: (*a*) $HgCl_2$ on SiO_2 gel at temperature 20 to 30°. (*b*) activated C at temperature up to 200°.

Methods to produce vinyl chloride from ethylene have also been developed. They involve the preparation of ethylene di-chloride and subsequent dehydrohalogenation either catalytically or with alcoholic alkali.



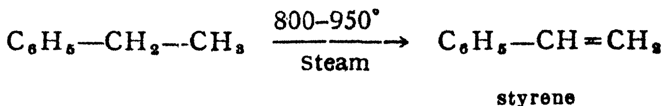
Recently vinyl chloride is obtained by the hot chlorin ation of ethylene.

Vinyl acetate—It is an important compound used in the preparation of copolymers. It is readily obtained by the action of acetylene on glacial acetic acid in presence of mercuric salt as a catalyst.



Styrene—It is second in technical importance to butadiene. It is obtained in very large quantities to be used as a secondary component to obtain the well-known copolymer Buna S, which has become a standard for the production of synthetic elastomers.

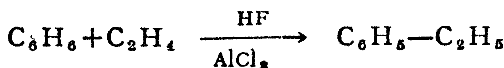
It is obtained from ethyl benzene by catalytic dehydrogenation :



$$C_6H_5—CH_2—CH_3 \xrightarrow[\text{Steam}]{800-950^\circ} C_6H_5—CH{=}CH_2$$

styrene

The dehydrogenation is effected at a lower temperature 700° in presence of Al_2O_3 as the catalyst. The ethyl benzene required in the above process is obtained by Ipatieff's method, from benzene and ethylene.



$$C_6H_6+C_2H_4 \xrightarrow[AlCl_3]{HF} C_6H_5—C_2H_5$$

Alternately, it may be produced from benzene and ethyl chloride in presence of $AlCl_3$.



$$C_6H_6+C_2H_5Cl \xrightarrow{AlCl_3} C_6H_5—C_2H_5$$

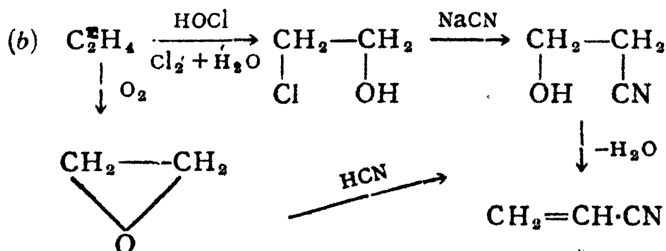
Styrene is a colourless liquid b. p. 143° with a characteristic pungent smell.

Acrylo-nitrile—This compound like styrene is used as a secondary component with butadiene in the preparation of the copolymer Buna N, (per bunan) : It is obtained on a large scale by one of the following methods, utilising either acetylene or ethylene as the starting material.



(*a*) $CH{\equiv}CH \xrightarrow{HCN} CH_2{=}CH{\cdot}CN$

acrylo nitrile



Acrylo-nitrile is a colourless liquid b. p. 177°; it possesses a pleasant odour.

Polymerisation

So far, we have discussed the production of primary materials *i. e.*, the monomers : butadiene, isoprene etc. belonging to the divinyl series and the styrene, vinyl chloride, vinyl acetate etc. which belong to the vinyl series. The next step is to convert them into the synthetic rubber materials, and it involves the conversion of simple molecules into greater macromolecules. The process by which a large number of monomers are linked up to form long open chains or macromolecules is called polymerisation; and further chains of varied length may be formed according to the conditions under which the polymerisation is effected. The product of such a polymerisation process is a polymer; it is a substance of very high molecular weight, whose molecules are built up of several recurring structural units.

Fundamentally, there are two types of polymerisation processes :—(1) The addition polymerisation, and (2) The condensation polymerisation.

Addition Polymerisation

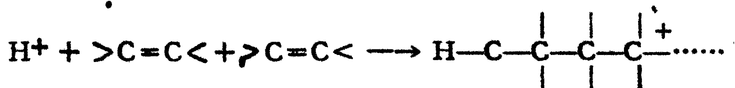
Addition polymerisation occurs when compounds belonging to vinyl series add to divinyl series *e.g.* vinyl chloride and butadiene respectively are treated with suitable catalyst under specified conditions. It is also called vinyl polymerisation and this involves the self-addition of the unsaturated molecules to each other, without the elimination of any small molecules. This can be expressed by the general formula :—



$$nR{-}CH{=}CH_2 \longrightarrow (R{-}CH{-}CH_2)_n{-}$$

In the absence of catalysts, the polymerisation of the vinyl compounds proceeds slowly. The process is greatly accelerated by heat, light and catalysts. The latter are varied in nature but can be classified into two types : (1) acid catalysts, acetic acid and mineral acids like HCl which supply a H^+ and (2) metallic Na and peroxides which supply a single odd electron.

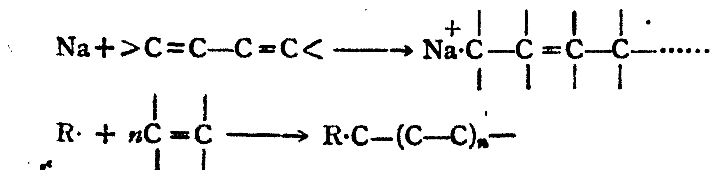
The H^+ attracts electrons and thus activates the double bond, which induces the self-addition *i.e.* polymerisation.



$$H^+ + {>}C{=}C{<} + {>}C{=}C{<} \longrightarrow H{-}\overset{|}{\underset{|}{C}}{-}\overset{|}{\underset{|}{C}}{-}\overset{|}{\underset{|}{C}}{-}\overset{|}{\underset{|}{C}}{}^{+}{-}\cdots\cdots$$

It is a chain reaction and the chain grows very rapidly and stops only when it comes into contact with a molecule, atom or radical which supplies an electron pair to the end carbon atom.

The second type of catalysts supply an electron which activates the double bond; The chain formation may be pictured as follows :—

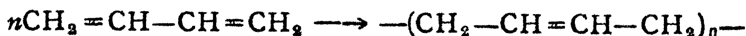


In this case, the chain grows till it comes into contact with another molecule which supplies a single electron to the end carbon atom.

In both the cases, the size of the polymer formed depends on (*a*) the number of monomer molecules, (*b*) the concentration of the catalyst and (*c*) the concentration of the molecules or substances present which can stop the chain growth. Substances called modifiers are also added to limit the degree of polymerisation and thus change the properties of the monomer molecules; lauryl mercaptan has been extensively used for this purpose.

The polymerisation process may lead to the production of *linear* polymers as described above. It may give rise to space or cross linked or three-dimensional polymers. The latter types of polymers are formed when two or more vinyl groups are present in the same vinyl derivative. They are produced by condensation polymerisation—an example of space polymerisation.

The synthetic rubber formation involves the vinyl polymerisation of a conjugated diene (which is a divinyl derivative). Thus in the presence of peroxides, a butadiene undergoes linear polymerisation.



$$nCH_2{=}CH{-}CH{=}CH_2 \longrightarrow {-}(CH_2{-}CH{=}CH{-}CH_2)_n{-}$$

The polymerisation may be carried out in gaseous, liquid or solid phase, but emulsion polymerisation has been found to be suitable for the production of synthetic rubbers. In this process, the different monomers in water are first emulsified with acid or soap or a wetting agent and stabilised by adding a protective colloid. The latex obtained is coagulated and processed in the same way as natural

rubber. The practical advantages of this process are: (1) it lends itself to careful temperature control, (2) it enables the addition of suitable modifying agents and catalysts, (3) it is much more rapid (requires a few hours only) and (4) it gives a more uniform product.

This method has therefore completely superseded the earlier polymerisation process.

Condensation Polymerisation

This is the second type of polymerisation and occurs with bi-or multifunctional molecules. Unlike the vinyl polymerisation, it proceeds with the elimination of small molecules. It may thus involve poly-esterification or polyamide formation, anhydride formation, aldolisation etc. Further it may lead to linear or space polymers; thus with bifunctional molecules, a linear polymer will be formed; while a space polymer with result when the polymerisation takes place between molecules which are multifunctional. The polymer formed by treating glycerol with phthalic anhydride is thus a space or three dimensional polymer.

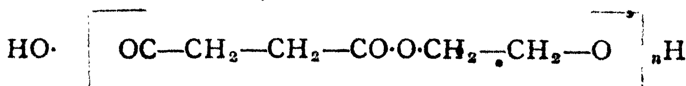
The condensation polymerisation has found many important technical applications. The formation of the synthetic fibres *e.g.* nylons, terylene etc. the synthetic alkyl resins, like glyptals, the Bakelite resins and the silicones, all involve condensation polymerisation reactions.

(*a*) Polyesterification—



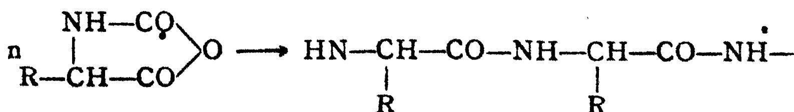
$HOOC{\cdot}CH_2{\cdot}CH_2{\cdot}COOH + HOCH_2{-}CH_2OH$





$HO{\cdot}\left[OC{-}CH_2{-}CH_2{-}CO{\cdot}O{\cdot}CH_2{-}CH_2{-}O\right]_nH$

(*b*) Polyamidation—A good example is the method of preparing polypeptides, due to Leuchs. It consists in heating N-carboxylanhydrides of α-amino acids.



These are linear or string polymers; an example of space polymerisation is given below:

$$HOOC{\cdot}CH_2{\cdot}CH_2{\cdot}COOH + HOCH_2{\cdot}CHOH{\cdot}CH_2OH$$
$$\downarrow$$

```
—OC·(CH2—CH2)—CO·O·CH2—CH—CH2·O·CO—
                        |
—OCH2·CH·CH2O·CO·CH2·CH2·CO
      |
      O
      |
      CO·(CH2—CH2)CO·O·CH2—CH—CH2—
                        |
                        O—
```

Vulcanisation

Vulcanisation is a process used to improve the elasticity and other physical properties of the rubber molecule. It consists in heating rubber with sulphur or sulphur compounds *e. g.* S_2Cl_2 (in CS_2). It is a time-temperature reaction and is greatly accelerated by the presence of sulphur containing organic molecules. The most efficient of such acceleration is zn-dimethyl-dithio-carbonate:

$$(CH_3)_2N{\cdot}\overset{\overset{S}{\|}}{C}{-}S{-}Zn{-}S{-}\overset{\overset{S}{\|}}{C}{\cdot}N(CH_3)_2$$

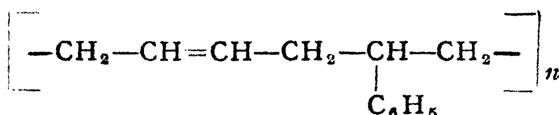
Thio-carbanilide and mercapto-benzthiazole are the other accelerators used for the purpose. It is believed that the process converts rubber which is a linear polymer, partly into a cross linked or space polymer.

```
—(CH2—CH=CH—CH2)n—        —CH2—CH—CH—CH2—
                                |   |
                 +Sx——>         S   S
                                |   |
—(CH2—CH=CH—CH2)n—        —CH2—CH—CH—CH2—
```

This process is also technically known as 'Curing'.

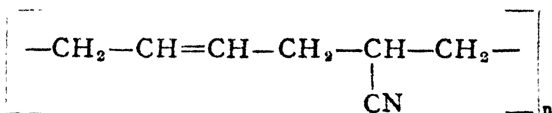
Some of the technically important synthetic rubbers are—Buna S, Buna N, and Duprene or Neoprene.

Buna S.—This is the most important of the synthetic rubbers and is produced in enormous quantities; it is also known as G.R.S. It is a copolymer obtained by polymerising a mixture of butadiene (75 per cent) and styrene (25 per cent). The copolymer is not a mechanical mixture of the two polymerised molecules, the molecules of both the monomers are incorporated into the long chains formed during the polymerisation. Thus Buna S has the repeating unit :—



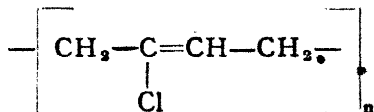
This rubber is mainly employed in the manufacture of tyres, and other mechanical rubber goods, as it possesses the greatest abrasion resistance.

Buna (Per bunan)—It is a copolymer and is obtained by polymerising butadiene containing 28-35 per cent of acrylonitrile. It possesses the repeating unit.



This type of rubber is characterised by its extraordinary resistance to swelling by petrol, oils and many other organic solvents.

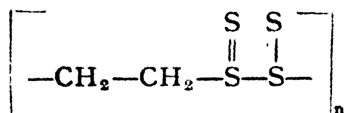
Duprene :—It is a linear polymer of chloroprene and has the repeating unit :



It is thermoplastic ; it need not be vulcanised by heating with sulphur. It is resistant to the action of oils, chemicals and organic solvents. It is not attacked by alkalies and acids below 50 per cent strength. The latter is a very valuable property.

Thiokol.—Recently, a new type of synthetic rubbers called the polysulphide rubbers has been developed : unlike the Buna S or

Buna N rubbers, these rubbers do not contain the fundamental structural pattern of the natural rubbers. The most important of these polysulphide rubbers is Thiokol. It is obtained by the action of an alkali polysulphide Na_2S_4 on ethylene dichloride. The two components are mixed in presence of a dispersing agent and the latex-like substance formed is coagulated with acid to a rubbery product. It probably contains the structural unit :



It is used for bullet proof fuel tank linings for air-craft. Large quantities are also used for protecting the cables. It is resistant to swelling by solvents and to abrasion.

Butyl rubber.—It is a still more recent development. It is a copolymer obtained by polymerising a mixture of isobutene and butadiene. The amount of the latter is very small, but is necessary to give a polymer with a certain amount of unsaturation necessary to effect vulcanisation. The polymerisation of isobutene alone gives polybutene rubbers which like hydrorubber cannot be vulcanised. Still another type of rubber is being developed: they are synthetic resins, mixed with plasticisers like tri-cresyl phosphate, dioctyl-phthalate. They show all the characteristic properties of rubber.

Lastly, it must be mentioned that attempts have been made to improve the qualities of the natural rubber itself; these attempts have led to the development of rubber derivatives like rubber hydrochloride or chlorinated rubber. Rubber hydrochloride is obtained by passing HCl gas into a benzene solution of rubber. This is being developed in Holland; it is more transparent and possesses a low moisture permeability. Hence, it is used for the preparation of photographic films (pliofilm) and as sheets for wrapping and packing. Chlorinated rubbers are obtained by the action of chlorine on rubber; they are hard, brittle powers, but are soluble in organic solvents. They are used for the preparation of paints and varnishes which are resistant to chemicals. A chlorinated rubber hydrochloride is also made on a commercial scale.

Synthetic Fibres

The natural fibres cotton, linen, wool and silk are built up of macromolecules and possess a few inconvenient properties. Attempts have been made therefore to develop synthetics in which these disadvantages have been more or less overcome. In one mode of attack of the problem, modifications of the natural macromolecules have been devised; in the other entirely new synthetic macromolecules, but modelled on the natural ones have been obtained from cheap materials of different types. Thus we have fundamentally two types of synthetic fibres :—

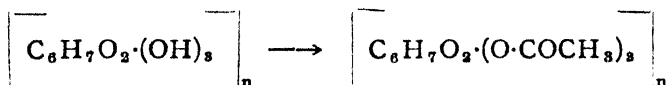
(1) Those derived from natural fibres which have cellulose base, protein base and others.

(2) Those derived from simpler molecules but modelled on the natural pattern : polyamides, polyesters, polyacrylonitriles etc.

Cellulose base fibres—The fibres belonging to this group have been conveniently subdivided into two classes: (*a*) estrons *i. e.* esters: cellulose nitrates (lower), acetate, butyrate and (*b*) rayons *i. e.* the regenerated cellulose : viscose etc.

The first artificial fibre was obtained from the nitrated cellulose derived from mulberry twigs. It was Chardonnet, the French chemist who wanted to imitate the silkworm. He produced fine threads by squeezing an alcohol-ether solution of cellulose nitrate through fine orifices. Cellulose nitrate is obtained by the nitration of cellulose with nitric acid in presence of a large excess of con H_2SO_4; the temperature is carefully controlled and the nitration is regulated to give only a dinitrated product with a nitrogen content of 10·5-12 per cent. The dinitrated product is also called pyroxylin : collodion is a solution of pyroxylin in alcohol-ether; the solution is forced through a spinnerette and the solvents are allowed to evaporate in warm air to give the threads. But the nitrated cellulose is inflammable and a method had to be developed to eliminate the nitro groups by treatment with alkali hydrosulphides. The denitrated thread resembled silk. However, the fibre did not find popularity and its manufacture was soon discontinued. The nitrocellulose fibre is an estron type of synthetic fibre. At present other esters of cellulose are being prepared and developed as estrons: the most important one is the cellulose acetate.

Cellulose acetate is obtained by acetylation of cellulose dissolved in acetic acid, with acetic anhydride; a small amount of H_2SO_4 is used as the catalyst. The acetylation of the cellulose molecule is accompanied by partial hydrolytic cleavage as is indicated by the fact that the cellulose acetate contains only 200 to 300 glucose units.



$$[C_6H_7O_2\cdot(OH)_3]_n \longrightarrow [C_6H_7O_2\cdot(O\cdot COCH_3)_3]_n$$

The fully acetylated product thus formed is treated with water enough to cause the desired degree of hydrolysis. The final product is then precipitated by throwing it into water. The acetate is dissolved in acetone and forced through a spinnerette and the threads dried by extrusion into warm air. The acetylation process is a costly one, and yet the acetate rayon is being produced in ever-increasing quantities. This is because the rayon has the following advantages :

(1) It is not inflammable; (2) it does not absorb much water when wetted; (3) it is more extensible and hence less susceptible to wrinkling.

Lastly, the cost is partly offset by the increase in weight by 40 per cent and further by the development of efficient recovery processes for the acetic acid and other solvents used in the process. Of the several processes known, the more important are :—Brewster process, Suida process, and the Bregeat process.

Celanese is the trade name for the cellulose acetate rayon obtained by dissolving the acetate in acetone and forcing it through fine orifices in a warm chamber. It is used for women's dresses.

Recently, other simple esters or mixed esters of cellulose are being developed as synthetic fibres. The simple esters are propionates, butyrates or stearates. Mixed esters in which some of the hydroxyl groups are esterified with one acid and others with a different acid are also known. They are more flexible than the acetate.

Cellulose ethers *e. g.* the ethyl cellulose, allyl cellulose and carboxymethyl celluloses are being developed as potential fibres or synthetic plastics.

Regenerated Cellulose—The rayons constitute the regenerated cellulose which is more reactive, unstretched and contains a larger amount of amorphous material. The rayons find extensive use as textile fibres. There are two important methods of preparing regenerated cellulose—(*a*) The Cuprammonium process, and (*b*) The Viscose process. Any form of cellulose *e. g.* cotton linters, wood cellulose with fibres too short to be useful for direct spinning, may be converted by one of these processes into a form capable of giving a thread, which is very valuable.

The Cuprammonium Process—In this process, the cellulose is first dissolved in an ammoniacal solution of cupric hydroxide. The filtered solution is forced through a spinnerette into a stream of water. The filaments thus obtained are then passed through a dilute solution of H_2SO_4, when the cuprammonium complex is destroyed and the cellulose in the filaments is regenerated. The filaments are finer than those of silk.

The Viscose Process—Cellulose is dissolved in CS_2 in presence of 15–20 per cent of NaOH at room temperature to form sodium cellulose xanthate (see p. 146). The solution is then ripened by allowing it to stand for some time; during this time, the desired amount of depolymerisation occurs. The solution is then forced through a spinnerette into a coagulating bath containing dilute H_2SO_4; in this way, threads of regenerated cellulose are obtained. If then solution is forced through a slit, the regenerated cellulose is obtained in the form of a sheet. This is known as the cellophane and is used as a protective and wrapping material. The threads of the regenerated cellulose find extensive use as textile fibres. A large proportion of the rayon produced today is made by the viscose process.

Protein base fibres.—They are also known as Azlons. There are two general classes of protein fibres: (1) those based on lactic casein, (2) those based on peanut protein.

Lanital is a synthetic fibre which represents the first type; it was developed by Feretti in Italy. A similar Dutch product is known as *Casolana.* The globular protein (casein) dissolved in water is forced through a die, and the filaments thus obtained are hardened by treatment with formaldehyde, which reacts with the free amino groups. The hardening improves the flexibility and resistance to water. The United States have developed two fibres *vicara* and

ardil ; they are derived from non-casein protein. Vicara is made from zein the protein of maize and ardil from the peanut protein. A soyabean protein fibre is also being developed. These protein fibres resemble wool in appearance and feel, and are capable of being fibres blended with wool. They, however, possess a low tensile strength when wetted, and hence are usually blended with other fibres (wool, nylon, viscose etc.).

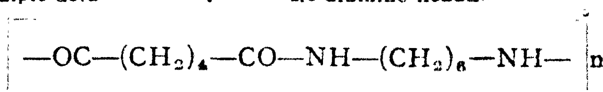
So far the synthetic fibres which are derived ultimately from large molecules of natural origin have been discussed. Since the end of the World War I, new fibres derived from simplemolecules by the processess of condensation —polymerisation have been developed. The most important of such fibres from commercial stand-point are Nylon, Saran, Terylene, Vinyon, Orlon etc.

Nylon.—It is the synthetic protein type of fibre. It is a linear polymer formed by the interaction of adipic acid and 1·6 diamino hexane (polyamide condensation). It cantains a long polyamide chain resembling the peptide chain of the protein molecule. It is the nylon '66'.



$$HOOC \cdot (CH_2)_4 \cdot COOH + H_2N \cdot (CH_2)_6 \cdot NH_2$$

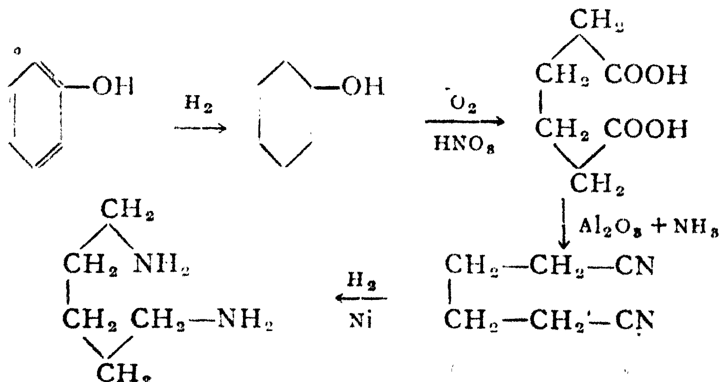
adipic acid 1.6 diamino-hexane



$$\left[-OC-(CH_2)_4-CO-NH-(CH_2)_6-NH- \right]_n$$

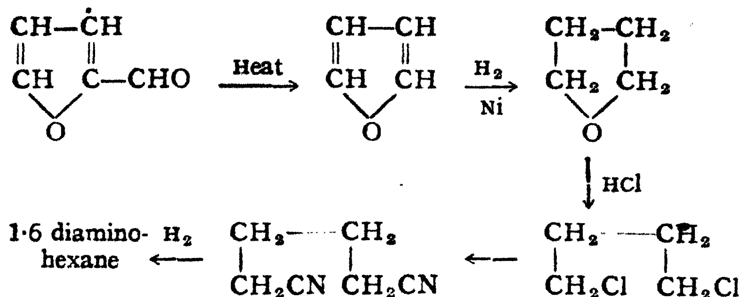
Both adipic acid and the diamine are obtained on a large scale from: (1) phenol and (2) furfural:

(1) *From phenol*—

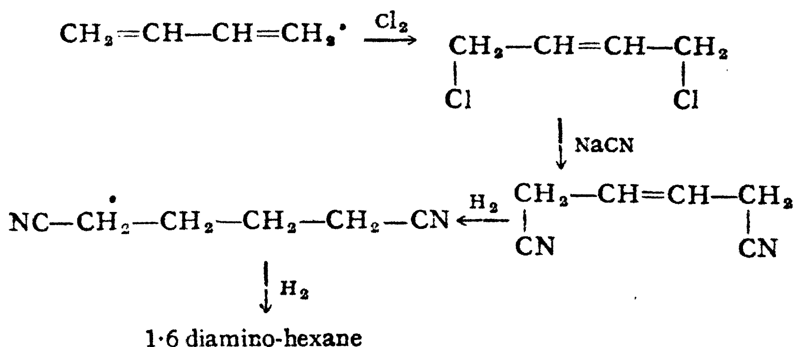


Instead of phenol, cyclohexane can be converted into adipic acid, by oxidation with nitric acid.

(2) *From furfural*—

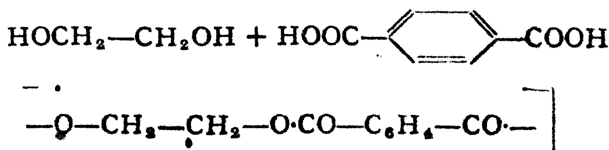


The diamine is also obtained from butadiene :



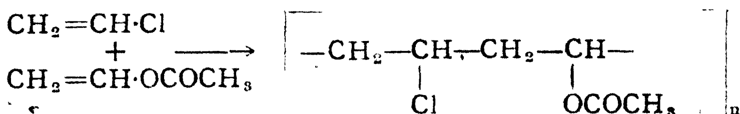
Nylon possesses a greater tensile strength than any natural fibre. It is also tough and shows good resistance to abrasion. Monofilaments can also be made which are suitable for the preparation of bristles for brushes.

Terylene.—It is a polyester fibre and is made from ethylene glycol and terephthalic acid; the esterification is effected in an autoclave.



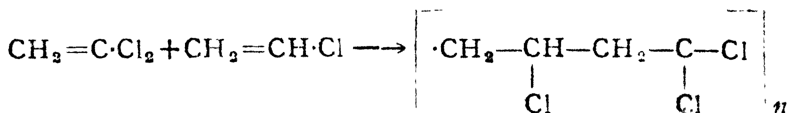
It is an I.C.I. product; it possesses high tensile strength and resiliency. It is also stable to hydrolytic reactions. In U. S. A. it is known as Dacron.

Vinyon.—This is the product of addition-polymerisation reaction. It is the co-polymer obtained by polymerising together vinyl chloride (88·95 per cent) and vinyl acetate (12-15 per cent).



This fibre is characterised by its remarkable resistance to acids, alkalies and other chemicals. But it is thermoplastic which limits its usefulness. However it finds use in the manufacture of felts.

Saran.—It is a copolymer of vinylidene chloride. $CH_2=CCl_2$ (90 per cent) and vinyl chloride (10 per cent).



It is suitable for dress materials and upholstery fabrics. It can also be used as substitute for hemp or long fibre paper. It is very resistant to acids and alkalies, but is attacked by ammonia.

Orlon and Vinyon N are synthetic fibres of growing use and commercial importance. Orlon is a polyacrylonitrile and the second is a copolymer of vinyl chloride and acrylonitrile. They possess certain advantage over Saran and similar Vinyl fibres. Recently, *acrylon* a new fibre has been put on the market by the Monsanto Chemicals Ltd. It is obtained by the polymerisation of acrylonitrile under suitable conditions.

Synthetic Plastics and Resins

The terms resins and plastics are sometimes used interchangeably, though they really represent two different types of compounds. They are tough substances capable of being moulded, *(they soften on heating) while the resins lack this property ;*

the former also possess a much higher molecular weight than the latter. But the demarcation is not sharp; a compound like polystyrene is a plastic above 65° and is a resinoid at room temperature. In the industry, on the other hand, the unfabricated article is referred to as the resin, while the fabricated product is called a plastic.

The natural resins are chiefly of vegetable origin and constitute the exudates from pine or fir trees; they occur alone or as mixtures with turpentine or other essential oils hence called oleoresins; they are few in number; they are: rosin, copal, damar and sandarac; they are also limited in their applications, their major use being in finishing composition, paints, varnishes, etc.

Shellac is a natural resin of non-vegetable origin; it is a product of secretion of the lac insect and finds use in lacquers.

The synthetic resins and plastics on the other hand, are the products of modern research and are not only more numerous than the natural products, but have found a much wider application. They have been arbitrarily classified as follows (based on their composition and source).

(*a*) Cellulose Plastics—nitrate and other esters and ethers.

(*b*) Formaldehyde Plastics—which can be subdivided into: (1) phenol formaldehyde, (2) ureaformaldehyde, (3) melamine formaldehyde resins, (4) ion exchange resins, and casein plastics.

(*c*) ALKYD RESINS.

(*d*) Polymer resins namely vinyl resins, acryloid resins, teflon, polystyrene and the silicones (organo-silicon polymers).

CELLULOSE PLASTICS—The first synthetic plastic was made from pyroxylin, cellulose di-nitrate. It is generally known by the name of celluloid; it is pyroxylin which is plasticised by the incorporation of camphor (2-20 per cent) into the material. It has found many applications *e. g.* in the manufacture of articles like films, spectacle frames, containers etc.

Recently, pyroxylin in a modified form has found a new use in the preparation of lacquer paints. The pyroxylin is heated under

pressure in contact with dilute acid when depolymerisation occurs and the modified form is more soluble in the usual solvents. Butyl acetate is an excellent solvent for such a modified pyroxylin, and is used to obtain a good lacquer.

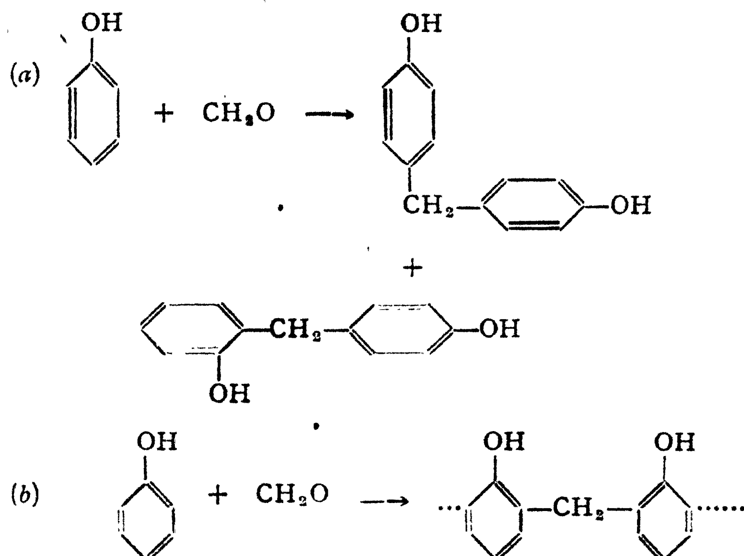
Cellulose-acetate plasticised by camphor is a very popular moulding plastic. It has led to the development of the new technique of injection moulding, because it can be subjected to heat and pressure without any hazard. It is believed that about two hundred parts made from this plastic are now used in an automobile. Airplanes are now provided with sheets of this plastic, instead of the fragile glass sheets.

A recent development has been to use plasticised mixed cellulose esters. The most common is the cellulose acetatebutyrate: it requires a smaller amount of the plasticiser, but the finished product is as suitable as the plasticised acetate. Still another development has been to employ the cellulose ethers suitably plasticised. The ethers are stable to alkalies and acids and are readily compatible with many placticisers; commercially, the methyl, ethyl and the benzyl ethers are made on a large-scale and used for the preparation of films and molded articles.

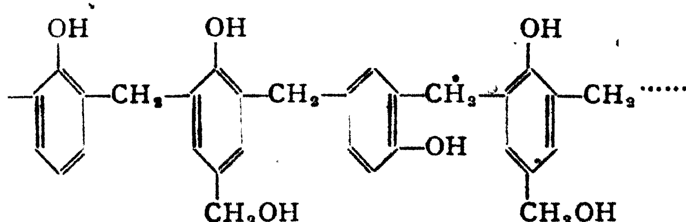
FORMALDEHYDE PLASTICS.—The cellulose plastics described above are thermoplastic. They are rigid at ordinary temperatures, but can be remolded by heat and pressure and the change is reversible. There is a second type of plastics called 'thermosetting plastics'; they are fusible but become permanently hard and infusible, under the influence of heat and pressure. The change from fluid to solid, in this type of resins is irreversible. The resins belonging to this type are: (*a*) phenol-formaldehyde resins, and (*b*) urea formaldehyde resins. The ion exchange resins are phenol-formaldehyde condensation polymerisation products.

Phenol-formaldehyde resins.—It was Bakeland who initiated the technical application of the simple reaction between phenols and aldehydes discovered by Baeyer. He prepared the first phenol formaldehyde resin—the Bakelite and thus started the modern fast expanding synthetic resin industry. He condensed phenol with formaldehyde. The reaction takes place in stages and the nature of the product formed depends on the experimental conditions.

Thus in presence of dilute acids, the Novolak type of condensation takes place:

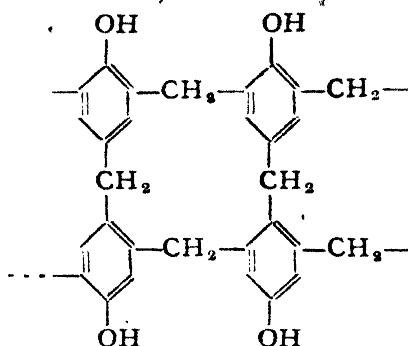


The Novolak type resin is a mixture of long chain polynuclear compounds of the diphenyl methane type of structure. (It carries no methylol groups). It is probably a *linear* polymer. Such Novolaks are used as intermediates for the preparation of the Bakelite type of resins. The Novolak resins may also contain another type of condensation product which carries methylol groups:



In this case, during the final molding, further condensation occurs between the methylol groups and a free ortho or para position of the phenol, to form a three dimensional or cross linked polymer.

In alkaline medium, the condensation goes further and a three dimensional polymer is formed containing the following linking:



It constitutes the Bakelite type of resins. This readily explains the conversion of the Novolak type of resins into the Bakelite ones, by moulding it under pressure and at high temperature.

In actual commercial practice, a Novolak resin in first made and is mixed with fillers like wood-flour and 12–14 per cent hexamethylene-tetramine; the latter heated on rollers is a source of both formaldehyde and the catalyst ammonia. The formaldehyde gives rise to new methylene bridges and thus forms cross-links giving the three dimensional polymer. No water is produced, since the Novolak resin used contains no CH_2OH groups. This is a great advantage, for the presence of water in molded materials greatly lowers the electrical insulating properties.

Bakelite resins are all three dimensional polymers and are all thermosetting resins. Hence in their preparation, phenols having three reactive positions must be employed *e. g.* phenol, recorcinol, *m*-cresol etc. On the other hand, if only the linear type of polymers Novolaks, are required, phenols with one of the ortho or para positions blocked by an inert group like CH_3, SO_3H etc. are used in the condensation process. The cast phenolic resins *e. g.* catalin gemstone etc. which have found many applications are also phenol-formaldehyde resins. However, they are obtained in a slightly different way. The mixture of phenol (1 mol.), formaldehyde (1·5-2·5 mol.) and NaOH or KOH is heated and the water formed is removed. It is then neutralised with lactic acid and vacuum-dis-

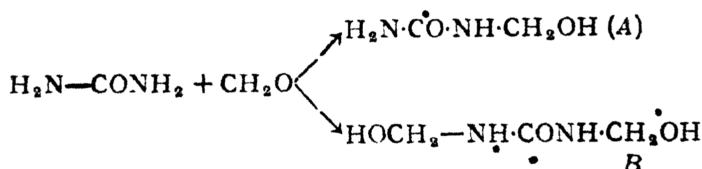
tilled to give a thick syrup. It is then mixed with glycerol and cast into moulds. They are chiefly used for the manufacture of decorative articles.

The phenol-formaldehyde resins find many applications: they are used for the manufacture of gears, protective coatings, pieces of furniture, and electrical equipment. They can be laminated with paper, leather, canvas and heat set, to obtain products with great resistance to impact. They are unsuitable for use in varnishes and paints, as they are insoluble in oils. Their disadvantage is that they are usually dark coloured and hence cannot be used for fabricating white or bright coloured articles.

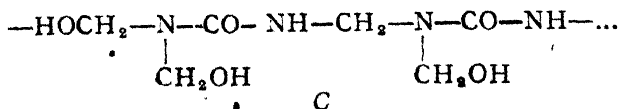
A great and important modification introduced in the manufacture of these resins, is the use of furfuraldehyde for formaldehyde. Furfuraldehyde is obtained by distilling the corncobs, oat-hulls, agricultural waste, with dil H_2SO_4. It is a highly reactive aldehyde and resinifies readily in presence of alkali.

Finally, new thermosetting resins are obtained by condensing formaldehyde with (*a*) urea and (*b*) with melamine.

Urea-formaldehyde resin.—Formaldehyde condenses readily with urea to form monomethylol (A) and dimethylol urea (B).



These condensation products may react further to form on heating a linear polymer (C) or a three dimensional polymer product (D).



```
—N—CO—N—CH₂—N—CO—N—CH₂—
 |      |      |
CH₂    CH₂    CH₂
 |      |      |
—N—CO—N—CH₂—N—CO·N—CH₂—
                 |
```

D

However, it must be admitted that the exact structure of these resins is not known. Urea is dissolved in aqueous formaldehyde below 10° and condensation is effected by heating the mixture to 130°. Water is removed under reduced pressure. The product is a colourless resin which is mixed with fillers *e.g.* wood flour and cellulose pulp and molded. Dyes may be used to give a coloured product. Urea-formaldehyde enamels are particularly valued, on account of their hardness, resistance to wear and stable colour.

Melamine formaldehyde resins.—These resins resemble very closely the urea-formaldehyde resins. Melamine is prepared by heating dicyandiamide under pressure; the latter is obtained from cyanamide by heating :

$$H_2N\text{—}CN \longrightarrow H_2N\text{—}\underset{\underset{NH}{\|}}{C}\text{—}NH\text{—}CN \longrightarrow C_3N_3(NH_2)_3$$

```
                                          N
                                         / \\
H₂N—CN ——→ H₂N—C—NH—CN  ——→   H₂N—C     C—NH₂
                ||                 N||     |N
                NH                    \   //
                                        C      melamine
                                        |
                                       NH₂
```

The resins are obtained by heating melamine with formaldehyde. Hexamethylol derivative with the structure :

```
                N
               / \\
(HOCH₂)₂N—C     C—N(CH₂OH)₂
          N||     |N
             \   //
               C
               |
            N(CH₂OH)₂
```

is first formed which then undergoes condensation-polymerisation through the reactive methylol groups to give the resin.

Ion exchange resins.—They are phenolformaldehyde resins containing acidic and basic groups. They are thus capable of an ion-exchange by virtue of the groups present. Thus the resins containing the groups SO_3H, COOH and CH_2—COOH, can exchange a cation while those containing the groups NH_2 and NH, can effectively exchange an anion or basic radical. The anion exchange resins are obtained by condensing formaldehyde with amines like *m*-phenylene-diamine and urea. The exchange resins are finding very valuable applications in modern industry. They are used in the demineralisation of water. They also provide a valuable means of recovering valuable materials from dilute solutions, from which, their extraction would be otherwise unprofitable. Thus alkaloids and amino acids can be separated by ion-exchange resins. Similarly inorganic impurities can be effectively removed by the application of a suitable ion-exchange resin.

Casein Plastics.—They are obtained by hardening with 4 per cent formaldehyde, casein articles which are pre-plasticised under pressure with plasticisers like glycerol, tri-cresyl phosphates etc. Casein is the principal protein of cow's milk; it is built up of many amino acids. The hardening process entails condensation of the amino groups of the amino acids with formaldehyde.

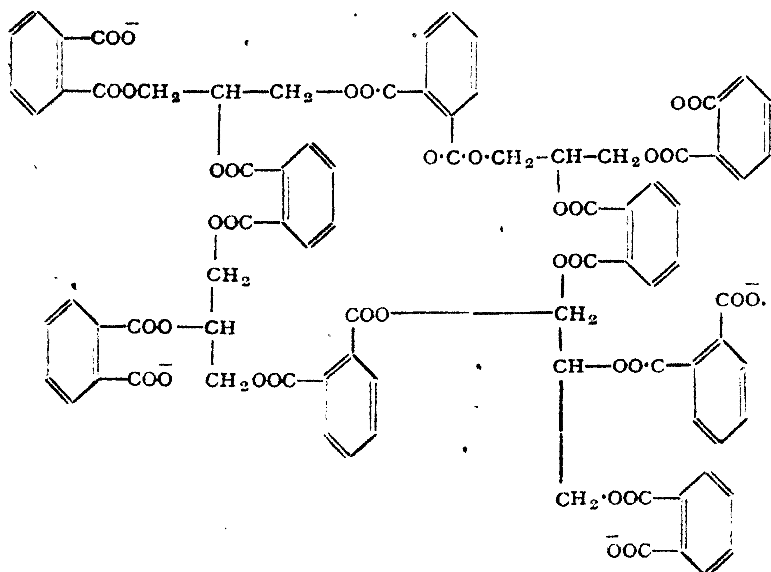


$$—NH_2+CH_2O+H_2N— \longrightarrow —NH—CH_2—NH—$$

But it is not known for some time whether formaldehyde reacts as a monomer or as the polymeric form $HOCH_2(CH_2O)_n{\cdot}CH_2OH$. Recent results show that the amount of formaldehyde used up is such as can be accounted for on the basis of paraformaldehyde.* The most important use of this plastic is in the manufacture of buttons and buckles.

Glyptals or alkyd resins. Structurally the alkyd resins are the polyester condensation products. They are formed by condensing polyhydric alcohols like ethylene glycol, glycerol etc. with polybasic acids or their anhydrides. The most commonly used acids are phthalic acid, isophthalic acid, succinic acid, tartaric acid and citric acid. They are three dimensional or cross-linked polymers. Thus

the typical glyptal resin obtained from phthalic acid and glycerol has the following structure.



They form a tough leathery mass at high temperatures, which hardens on prolonged heating. They find use as binding material for asbestos, cement etc. They are combined with natural resins or other synthetic resins and with drying ils to form the mixed plastics like *paralacs.* They are chiefly used in the preparation of paints, lacquers etc.

It is of great practical significance that both thermosetting and thermoplastic types of resins of a great variety can be obtained by a suitable variation of the acid and alcohol component.

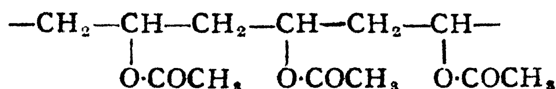
Polymer Resins

A large number of technically important resins are known which are formed by addition polymerisation under suitable conditions, of a number of synthetic monomers. The typical ones are : vinyl resins, acryloid resins, polystyrene, teflon and the silicones. The chemistry of the formation of these resins is briefly mentioned here :—

Vinyl Resins—They are of three kinds : (*a*) polymers of vinyl acetate, (*b*) polymers of vinyl chlorides and (*c*) polymers of vinylidene

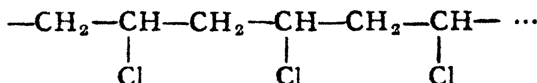
chloride. They are all thermoplastic and range from rubber-like products to hard and brittle ones.

Vinyl acetate resins—Vinyl acetate is obtained on a large scale by the catalytic addition of acetic acid to acetylene. The most efficient catalyst is mercury acetyl, sulphuric acid. It is then polymerised by the emulsion technique to the acetate resin. The polymerisation is catalysed by peroxide, ozone or ultra-violet light. The vinyl acetate molecules are linked head-to-tail to form a linear polymer.



They are not suitable for molding compositions, but are used for the preparation of lacquers and adhesives.

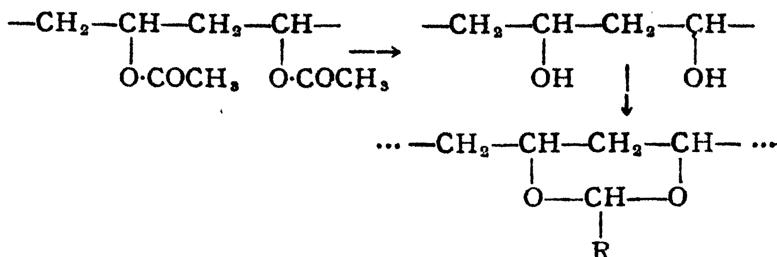
Vinyl chloride resin—The monomer vinyl chloride is obtained in two ways: (*a*) by the catalytic addition to HCl to acetylene and (*b*) by partial dehydrohalogenation of ethylene, dechloride, which is obtained from ethylene and chlorine. Vinyl chloride thus obtained is polymerised by a method analogous to that used in the polymerisation of the acetate. The resin also possesses analogous structure.



These resins are resistant to water and are thermoplastic. It is also used as a rubber substitute under the name Koroseal. Solutions of the resin in suitable solvents find use in the preparation of varnishes.

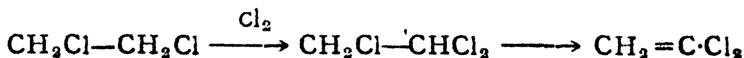
Resins of desirable properties have been obtained by the copolymerisation of vinyl acetate and vinyl chloride, co-polymers containing a larger proportion of the chloride yield, strong products which are suitable for molding compositions and for surface coatings. They also find use as synthetic fibres.

Lastly, modified resins are obtained by chemical treatment of the polyvinyl acetate resin. The latter on hydrolysis gives the polyvinyl alcohol resin, which can be further condensed with aldehydes to give polyvinyl acetal resins.



Both the products find specialised applications. The alcohol resin which is water soluble is used in the preparation of gloves to protect against organic solvents. The resin is used to prepare the safety glass by sandwitching it between glass plates.

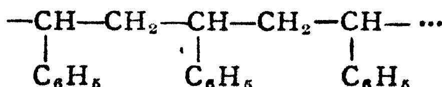
Vinylidene chloride resins.—Vinylidene chloride is obtained from ethylene di-chloride as follows :—



It is then polymerised by one of the usual techniques to give the resin.

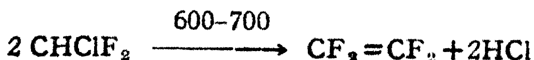
Polystyrene.—This resin is obtained by the emulsion polymerisation of styrene. The polymerisation is catalysed by peroxides like dibenzoyl–peroxide. Styrene is obtained on a large scale from benzene and ethylene.

The structure of polystyrene is—



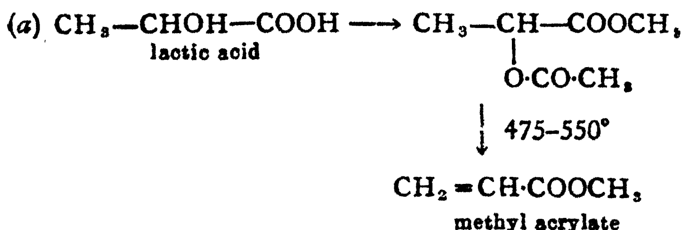
Polystyrene is used in injection molding. It has a high tensile strength and does not absorb moisture. Its high dielectric constant makes it very useful in radio insulation. It is also very clear.

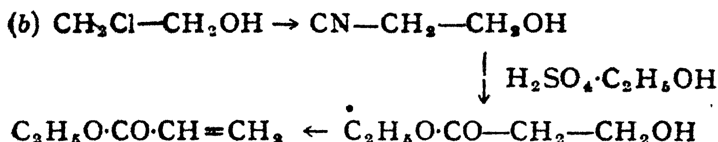
Teflon is the polymer of tetrafluoro–ethylene. The latter is obtained by pyrolysis of chlorodifluoro methane, which is obtained by the action of HF on $CHCl_3$ in presence of $Sbcl_5$



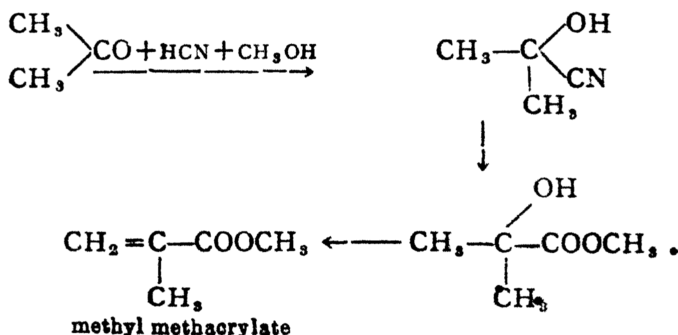
Teflon possesses great resistance to solvents and to boiling acids even to aqua regia. It also possesses great thermal stability.

Acryloid Resins.—These are the polymers of acrylic acid and its derivatives. The methyl ester is most commonly used. It is obtained now in two different ways: (*a*) from lactic acid and (*b*) from ethylene chlorohydrin.





A derivative of acrylic acid which is much used is the methyl methacrylate, it is obtained from acetone. HCN and H_2SO_4 in presence of CH_3OH.



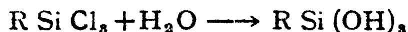
The polyacrylates are characterised by their transparency and hence are used in the manufacture of safety glass. The methyl methacrylate resin is the nearest approach to an organic substitute for glass. The perspex, polymer of methyl methacrylate is such a product. These resins are also finding applications as protective coating etc.

Silicones

The silicones are the organosilicon polymers. They represent the condensation polymers obtained by heating alkyl-silane-diols $R_2Si(OH)_2$ and alkylsilanetriols $RSi(OH)_3$. The latter are obtained as follows—



$$SiCl_4 + R\text{-}MgX \longrightarrow R\,SiCl_3 + R_2\,SiCl_2 \text{ etc.}$$



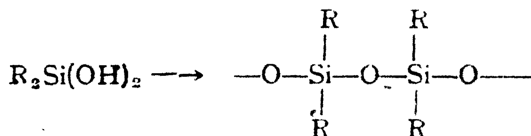
$$R\,Si\,Cl_3 + H_2O \longrightarrow R\,Si\,(OH)_3$$



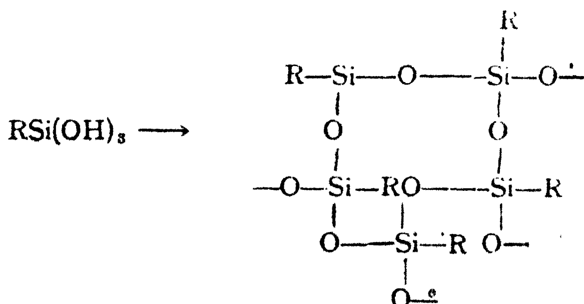
$$R_2\,SiCl_2 + H_2O \longrightarrow R_2\,Si\,(OH)_2$$

Recently Rochow has developed a vapour phase method of obtaining the chloro-silicanes by the action of alkyl halides, especially the chlorides, with silicon at high temperature, in presence of catalysts like Cu or Ag., This eliminates the use of the costly magnesium metal. The principal products are $CH_3Si\,Cl_3$, $(CH_3)_2$—$Si\,Cl_2$ and $(CH_3)_3Si\,Cl$.

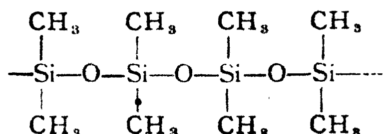
The chlorosilicanes, on hydrolysis give rise to the silanols *i.e.* the silane diols or triols which undergo condensation polymerisation to form both the linear and cross-linked polymers. The diols give the linear polymers which are siloxanes.



The triols yield the cross-linked polymers :



Copolymerisation of the diols and triols give rise to polymers containing a few cross-linkages. These cross-linked polymers possess a structure which resembles silica and are thus very stable to heat and chemical reagents. The silcones range in properties from heat stable liquids through lubricants which are resistant to hard solids which are infusible. They thus promise to be useful in a number of ways. They are thus used in the manufacture of water-proofing paper, textiles etc. The 'bouncing putty'—a silicon composition is being used to replace rubber in the cores of golf balls. Silicone rubber—a linear polymer has definite advantages over natural rubber, in being more stable to heat and to chemicals and yet being flexible even at low temperatures. It has the structure:



The silicone rubber can be vulcanised by treatment with benzoyl peroxide to give a product which possesses suitable properties over the wide range —100—+500°F.

INDEX

(Volume I—Parts 1 and 2)

B

C

D

E

H

L

M

N

O

P

Q

R

T

W

X

Z